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CONTENTS

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12 Inheritance of sex in vitis rotundifolia by L. R. Detjen

13 Biological investigation of sphenophorus callosus oliv. by Z. P. Metcalf

14 Repair of bone in domestic fowl by B. F. Kaupp

15 A monograph on trembles or milksickness and white snakeroot by F. A. Wolf and others

16 Clover stem rot by F. A. Wolf and R. O. Cromwell

17 The limits in hybridization of vitis rotundifolia with related species and genera by L. R. Detjen

18 Some F_1 hybrids of vitis rotundifolia with related species and genera by L. R. Detjen

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JANUARY, 1917 TECHNICAL BULLETIN 12

NORTH CAROLINA

AGRICULTURAL EXPERIMENT STATION

CONDUCTED JOINTLY BY THE

STATE DEPARTMENT OF AGRICULTURE

AND THE

NORTH CAROLINA STATE COLLEGE OF
AGRICULTURE AND ENGINEERING

RALEIGH AND WEST RALEIGH

INHERITANCE OF SEX IN VITIS ROTUNDIFOLIA

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FOREWORD

Vitis rotundifolia, the Muscadine grape, has been the subject of investigation by the Department of Horticulture of the North Carolina Agricultural Experiment Station for a number of years, with a view to the determination and the discovery of facts relating to its origin, its characteristics, and the laws which govern their transmission. The results of such research should serve as an addition to our plant-breeding knowledge and help to form a substantial basis for the improvement of this class of grapes.

The results which have been accomplished are set forth in the following bulletins:

1. "Scuppernong and Other Muscadine Grapes: Their Origin and Importance," by F. C. Reimer, Bulletin No. 201, N. C. Agr. Exp. Station. 1909.

2. "Self-Sterility of the Scuppernong and Other Muscadine Grapes," by F. C. Reimer and L. R. Detjen, Bulletin No. 209, N. C. Agr. Exp. Station. 1910.

3. "Breeding Rotundifolia Grapes: A Study of Transmission of Character," by F. C. Reimer and L. R. Detjen, Technical Bulletin No. 10, N. C. Agr. Exp. Station. 1914.

The present bulletin deals with the problems of transmission of sex and constitutes the fourth contribution to this series of reports of work accomplished.

J. P. Pillsbury,
Horticulturist.

TABLE OF CONTENTS

PAGE
I. Introduction 5
II. Morphological differences in the flowers of Vitis rotundifolia 6
III. Introduction of hermaphrodite vines with upright stamens 7
IV. Investigations regarding the inheritance types of flowers in Vitis rotundifolia 16
V. Correlation between type of stamen and character of pollen, and between size of flower cluster and character of pollen 25
VI. Similarity between flower clusters on hermaphrodite vines with upright stamens and those on staminate vines 28
VII. The probable origin of the Hope vine 31
VIII. Summary 34
IX. Literature cited 35
X. Plates.

INHERITANCE OF SEX IN VITIS ROTUNDIFOLIA

FIRST REPORT

By L. R. Detjen, Assistant Horticulturist.

I. Introduction

All commercial varieties of rotundifolia grapes of the present day are self-sterile. Because of this fact, staminate vines are required in the vineyards, especially true of large commercial vineyards, in order to produce profitable crops. Just what proportion of the vines should be staminate in order to insure maximum crops is at present an open question. As recommended by Reimer and Detjen (1)[*] one staminate vine should be planted for every eight fruit-bearing vines. This means that one vine out of every nine in the vineyard will bear no fruit, or, in other words, eleven and one-tenth per cent (11.1%) of the vineyard will be unproductive as far as fruit production is concerned. By planting staminate vines in the vineyard, land which is capable of producing fruit is given over to the production of pollen alone. Indispensable as pollen is, it is not the ultimate object of the fruit grower, and hence that land which is occupied by the staminate vines represents a direct loss.

By replacing each staminate vine with one which bears both pollen and fruit on the same vine, not only would pollen for cross-pollination be produced, but also a crop of fruit. Hence, if every ninth vine in the vineyard could thus be suddenly transformed from an indirect to a direct producer, the proceeds of the land might be increased eleven and one-tenth per cent.

If it were possible to replace all of our fruit-bearing varieties with self-fertile vines, ideal conditions respective to pollination would prevail in the vineyard. Each pistil in the flower would be surrounded by an abundance of pollen, and pollination itself would become independent of insect activities. Hence the crops of fruit would become more regular, irrespective of seasonal and climatic conditions, and the financial returns might be increased correspondingly.

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[*] Reference to cited literature is made by number.

II. MORPHOLOGICAL DIFFERENCES IN THE FLOWERS OF VITIS ROTUNDIFOLIA

Grape flowers can be divided into three general classes or types:

1. The perfect hermaphrodite flower.
2. The staminate or "male" flower.
3. The imperfect hermaphrodite or "female" flower.

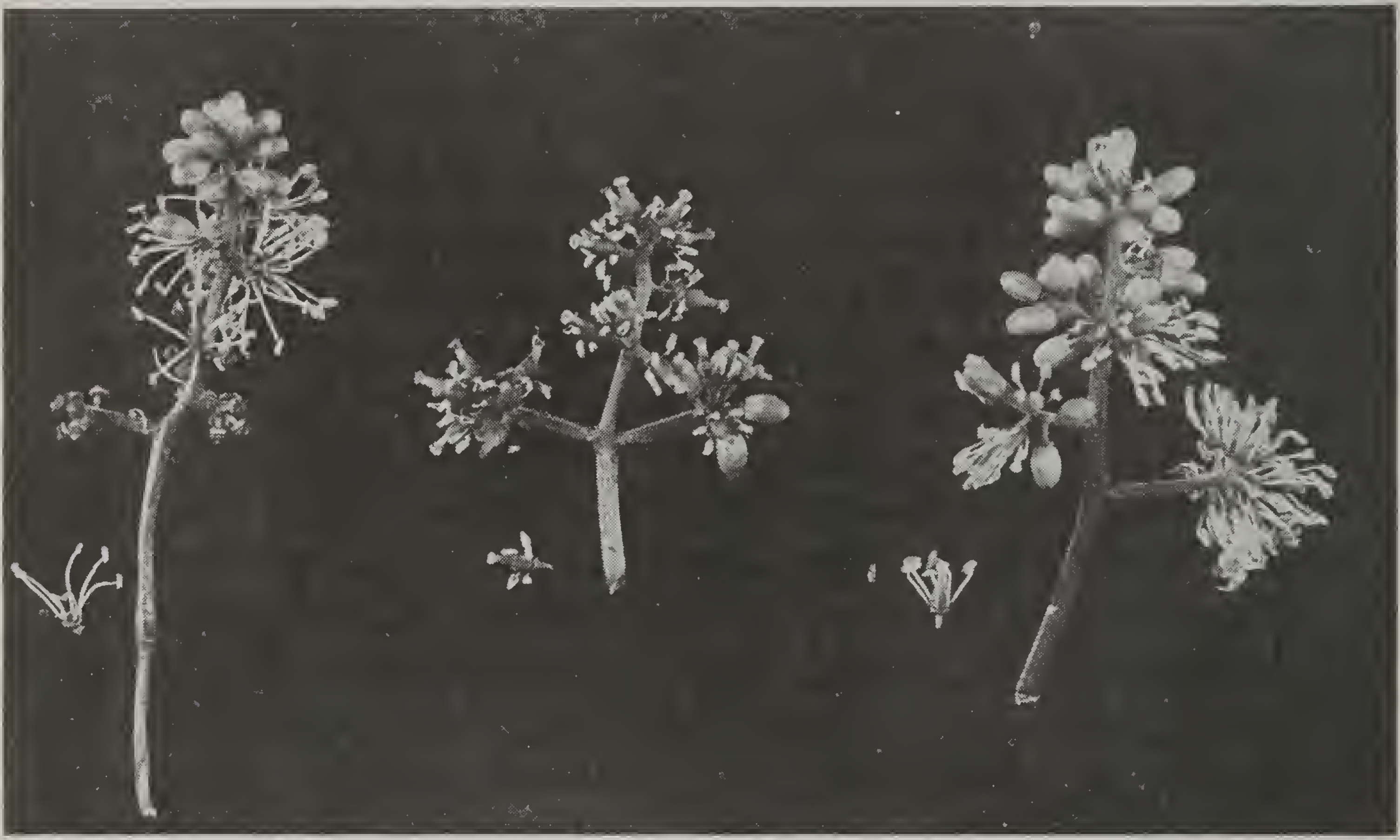

FIG. 1.—Three different types of flower clusters and flowers; on the left, staminate; in the middle, hermaphrodite with reflexed stamens; on the right, hermaphrodite with upright stamens. Natural size.

These types may be briefly described as follows:

After the petals, or "caps" as the united petals of a grape flower are called, have fallen, the perfect hermaphrodite flower consists essentially of a normal pistil and five or more upright stamens. Each pistil is functional and under proper conditions is capable of setting and developing a normal fruit. The stamens are strong and healthy. The filaments equal or exceed the combined length of the ovary, style, and stigma. They are stout and fairly symmetrically developed about their longitudinal axes. At the base of the ovary and between the stamens are situated the nectar glands, which secrete nectar, attractive to insects.

The staminate or "male" flowers are hermaphrodite flowers in which the pistils have been almost or wholly suppressed. It is almost superfluous to say that such flowers never produce fruit.

The imperfect hermaphrodite flowers are hermaphrodite flowers which are abnormal in one or more of the floral parts. In this type of flower the pistil has become more strongly developed by an enlargement of the ovary, a corresponding thickening of the style, and an enlargement of the stigmatic surface. The stamens have undergone a marked transformation in both their form and usefulness. The filaments no longer stand truly upright, as do those in the normal flower, but are more or less recurved or reflexed. According to Dorsey (2), this is due to the unsymmetrical development about their longitudinal axes. In length and in diameter they have suffered material degeneration. Very often their length is considerably less than the greater diameter of the anthers, and their transverse diameter has been so reduced that the filaments bear very much of a resemblance to fine, silken threads. The pollen that is produced by these stamens, instead of being plump and smooth, appears shrunken under the microscope [Reimer and Detjen (1)], and, when seen in quantity, very much resembles a heap of dented and collapsed tennis balls. It is needless to say that such pollen is unviable under the best of conditions, and therefore absolutely worthless. Hedrick and Anthony (3) state that many varieties of grapes with the reflexed type of stamen produce some normal viable pollen grains among the usual misshapen and abortive kind. Such mixed pollen may yet be found with some varieties of rotundifolia grapes; but, to date, many varieties and seedlings of this species have been examined and no normal pollen has ever been found associated with the reflexed type of stamen. The nectar glands that are found in the flowers with the reflexed type of stamen are well developed; they secrete nectar and emit a very fragrant odor which is attractive to many insects.

Such, in general, are the characteristics of the three types of flowers in the genus *Vitis*. Intergrading types are often found, but these will receive special consideration elsewhere.

III. INTRODUCTION OF HERMAPHRODITE VINES WITH UPRIGHT STAMENS

In the Summer of 1910, while the study of self-sterility of Vitis rotundifolia was in progress, Professor F. C. Reimer (1) discovered a wild vine with upright stamens growing in a plum thicket about one mile from the city of Raleigh, N. C. At the time of the discovery the vine bore a fairly good crop of fruit and, although late in the season, a few flower clusters. It was the flowers on these clusters that commanded attention.

Bagging experiments conducted immediately upon the discovery of the vine gave results indicative of self-sterility. The more extended work of the following two seasons gave similar results.

Although the data obtained in the work of these three seasons pointed very strongly toward self-sterility, a belief in the self-fertility of the vine persisted and evidence secured in later years substantiated the correctness of this supposition.

The strongest evidence that was available in favor of the self-fertility of the vine was the fact that when it was discovered it bore a fairly good crop of fruit. While none of the flower clusters that were bagged in the two succeeding years set fruit, neither did the flower clusters that grew out in the open produce any berries, although exposed to all chances for cross-pollination. Branches were also bagged and cross-pollinated with pollen from a light-colored staminate vine in order to test out the assumption that with this vine cross-pollination is absolutely necessary, but no fruit developed in any of these bags.

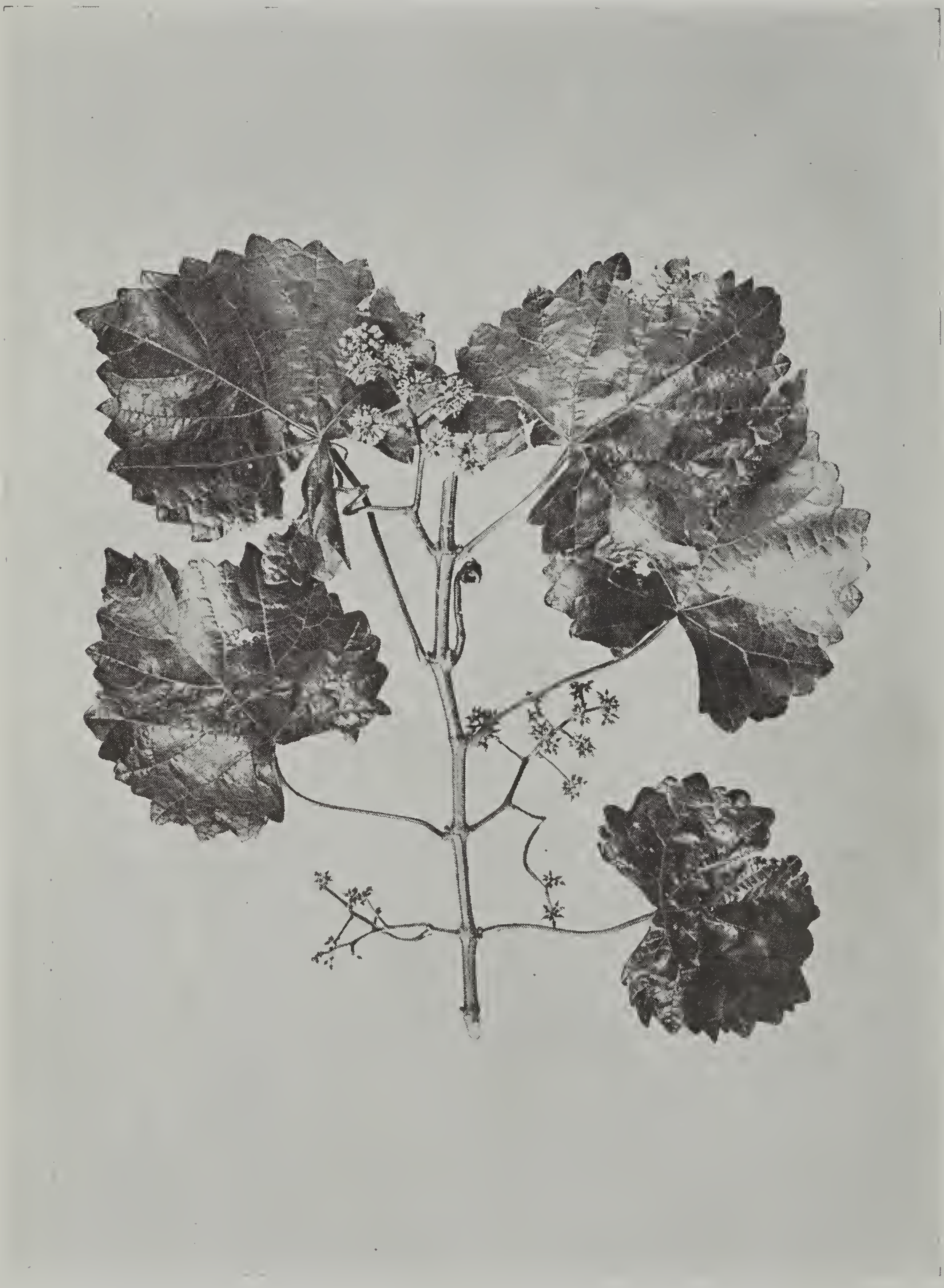

Fig. 2.—Lower section of a flowering branch of Hope. The two lower clusters have shed their stamens recently while the upper cluster is in full bloom. Reduced.

All of this evidence pointed not toward sterility because of defective pollen, but toward partial sterility, probably due to imperfectly developed pistils. The fact, however, that at one time a good crop of fruit had been produced was sufficient evidence that some day, perhaps, the vine might again return to fruitfulness, and this, as we shall see, has since happened.

In the meantime, as this vine was so full of promise, it was called "Hope," in commemoration of the hope and desire to find a self-fertile vine which might become the founder of a new race of rotundifolia grapes.

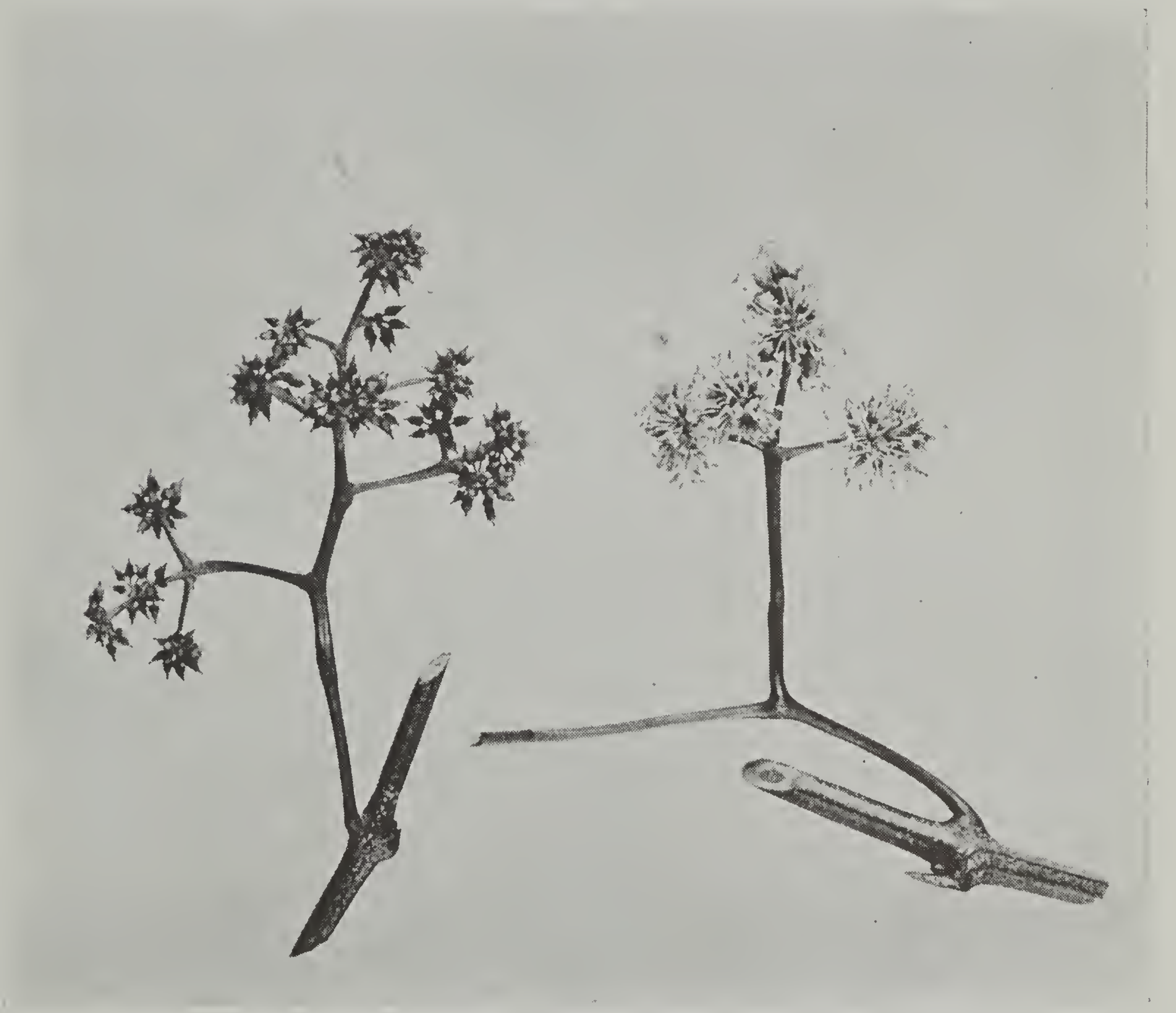

Fig. 3.—Two clusters of Hope. The cluster on the left is just beyond the blooming stage; note the number and the character of the pistils. The cluster on the right is in full bloom; note the upright stamens and the character of the pistils. Natural size.

Two other self-fertile seedlings, one a light-colored rotundifolia vine, origin not mentioned, and the other a dark-colored vine, a cross between Eden and a staminate vine, are reported by Husmann and Dearing (4).

These three vines are the only original ones mentioned as bearing flowers with upright stamens and with normal viable pollen. To these we look with confidence for the introduction and development of self-fertility among our varieties of rotundifolia grapes.

Description of the Hope vine; Floral Characters.—In general, the vine characters of Hope are typical of such as are found in the rotundifolia species. The plant is only moderately vigorous and is subject to the ravages of Black Rot (*Guignardia bidwellii*) in about the same degree as is the variety, Scuppernong. The main difference between this and other vines of the same species, however, lies in the floral characters. The flowers generally have neither the suppressed pistils of the staminate vines nor the recurved stamens of the common fruit-bearing vines, but they possess a combination of upright stamens with an abundance of healthy viable pollen, similar to that of typical staminate vines, and fairly well developed pistils, which upon fertilization are generally capable of developing into normal fruit. These pistils are not as large as those of other fruit-bearing varieties, neither is the stigma so well developed (Plate I, Fig. 2); still, the pistils are functional, and average sized fruit crops are often produced.

Occasionally, on some poorly nourished branch, which generally may be found on the lower or central portion of the vine, flower clusters are observed with some or all of the flowers representing various stages of development from the totally suppressed pistils of staminate flowers to the fairly well developed pistils of perfect flowers on other parts of the vine.

The filaments of the stamens in all of the flowers of this vine have the same strength, diameter, and upright position as do those in flowers of staminate vines. In short, the flowers of this vine might be termed staminate flowers whose pistils have reached a point in regeneration where they have become functional.

While the Hope vine is not as prolific in flower clusters as are some of our best staminate vines, nevertheless, it is quite representative of the average staminate vine. One or two flower clusters are usually produced on a cane, while three and four, although less often observed, are not at all of rare occurrence. Frequently, also, especially when the vine grows vigorously, many branches are produced which are absolutely barren.

The flower clusters of this vine are of fairly large size, considering the species. In fact, they are larger than those from the average staminate vine, and from external appearances, before the flowers begin to bloom, the clusters are seen to closely resemble those from a fairly good staminate vine. The number of buds on the cluster varies considerably, just as do those from all other vines. Countings made in the field place the number of buds per cluster anywhere between 100 and 300. Our best staminate vine produces clusters with buds ranging in number all the way from 200 to 384. It is probable that none of the fruit-bearing vines with reflxed stamens* produce clusters with more than 120 flowers, since the greatest number of flowers per cluster that has ever been recorded is 113. The vine which bore this cluster is a white fruited rotundifolia seedling found growing wild in the woods near West Raleigh, N. C.

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*Eden and San Monta are here excepted, because the former is considered a Munsoniana hybrid and the latter, supposedly a hybrid with a bunch grape, can probably be traced to a similar origin.

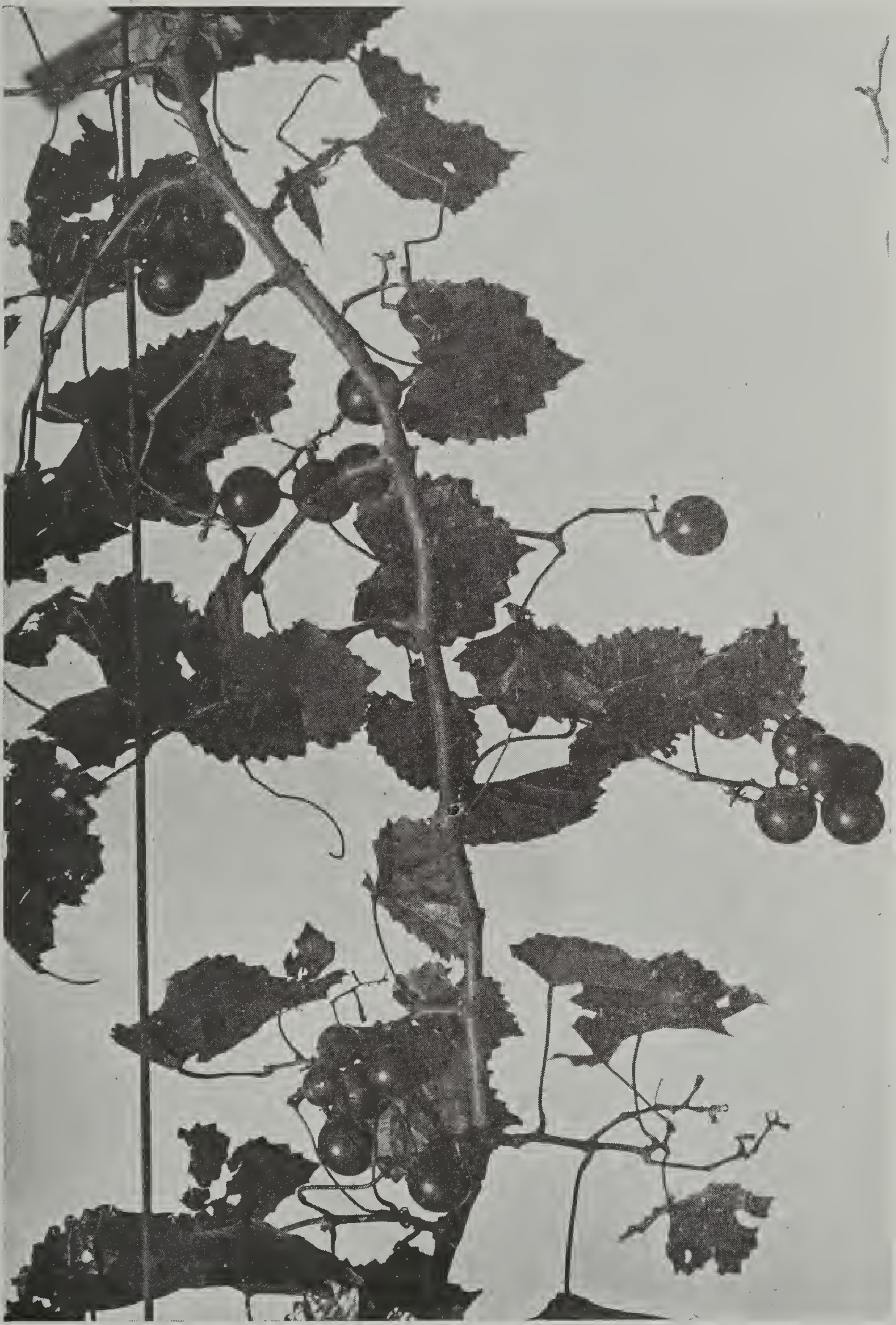

FIG. 4.—A typical fruiting branch from the Hope vine. Note the small fruit clusters. Reduced.

The fruit clusters of Hope are very small; one, two, three, and four berries usually constituting the average cluster. One would think that a cluster bearing from 200 to 300 flowers ought to produce a fairly good-sized fruit cluster, but, contrary to expectation, only a very few berries

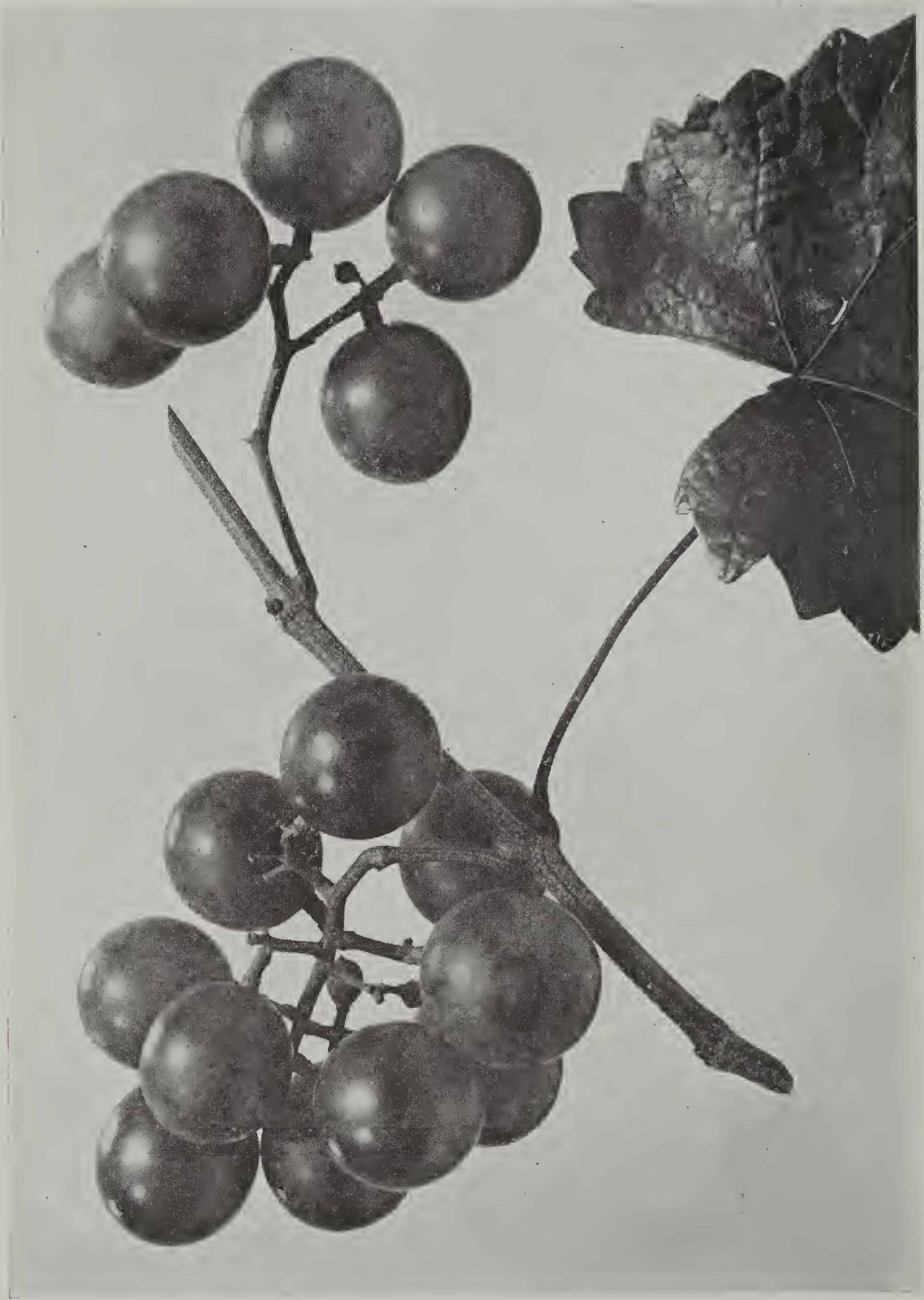

Fig. 5.—The largest cluster of fruit that was ever found on the Hope vine. The flowers of this cluster were not protected from cross-pollination. Natural size.

ever set on each of these clusters. The largest bunch of berries that was ever observed on this vine consisted of only ten berries, while the largest bunch of rotundifolia grapes ever observed by the writer was composed of thirty-five berries.* The number of berries per cluster varies with the general care of the vine.

The number of fruit clusters on the vine depends on many factors, among which the actual number of flower clusters produced, the degree of development of the pistils, and the amount of shedding that takes place soon after the flowers are fertilized are important items and demand consideration.

Self-fertility of Hope Vine.—In order to determine the exact status of the Hope vine in regard to the question of self-fertility, the following work was carried out:

In 1910, as soon as the vine was discovered, although this happened late in the season, two flower clusters which were still in bud were observed and immediately bagged. These bags were left undisturbed until all of the buds had bloomed, and later, upon examination of the contents of the bags, no fruit was found to have developed. The other parts of the vine bore an average crop of fruit which was produced from the normal flowers of the season.

In 1911 the experiment was repeated and, because of a greater supply of flower clusters, thirty-five branches were bagged and left to their fate. Not one of these thirty-five branches produced as much as a berry, but neither did the other parts of the vine bear any fruit.

In 1912 ten more branches, with two or more clusters to the branch, were covered. Although the vine bloomed profusely, no fruit was produced that year either in the bags or on the exposed branches of the vine.

In order to determine whether this vine failed to set fruit because of an existing uncongeniality between the pistils and the pollen of the same vine, or because of the lack of viable pollen, nineteen branches were bagged in 1911 and cross-pollinated with fresh pollen from a light-colored staminate vine. This pollen, although from a staminate vine, had no more effect on the setting of berries than did the pollen from the same vine, for no fruit was produced in the bags. The pistils, therefore, seemed to be faulty and incompetent to set fruit at times, regardless of the kind and source of pollen.

In 1913 only two berries were observed on the whole vine, and in 1914 only a handful, less than a dozen berries, constituted the total crop of fruit.

These negative results pointed very strongly toward sterility of the vine. It seems that for some unknown reason the vine appeared to have reverted all at once from a fruit-bearing vine to one that functions essentially as a staminate vine.

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*In 1914 a Luola vine produced an exceedingly heavy crop of fruit, and among the many large clusters one was found composed of 35 berries.

In the spring of 1911 a layer of this vine was transferred to the College grounds in order to have the vine under better observation and also to preserve the strain in case destruction should threaten the original vine. This precautionary measure was indeed a fortunate one, because during the spring of 1916 the original vine was destroyed by fire.

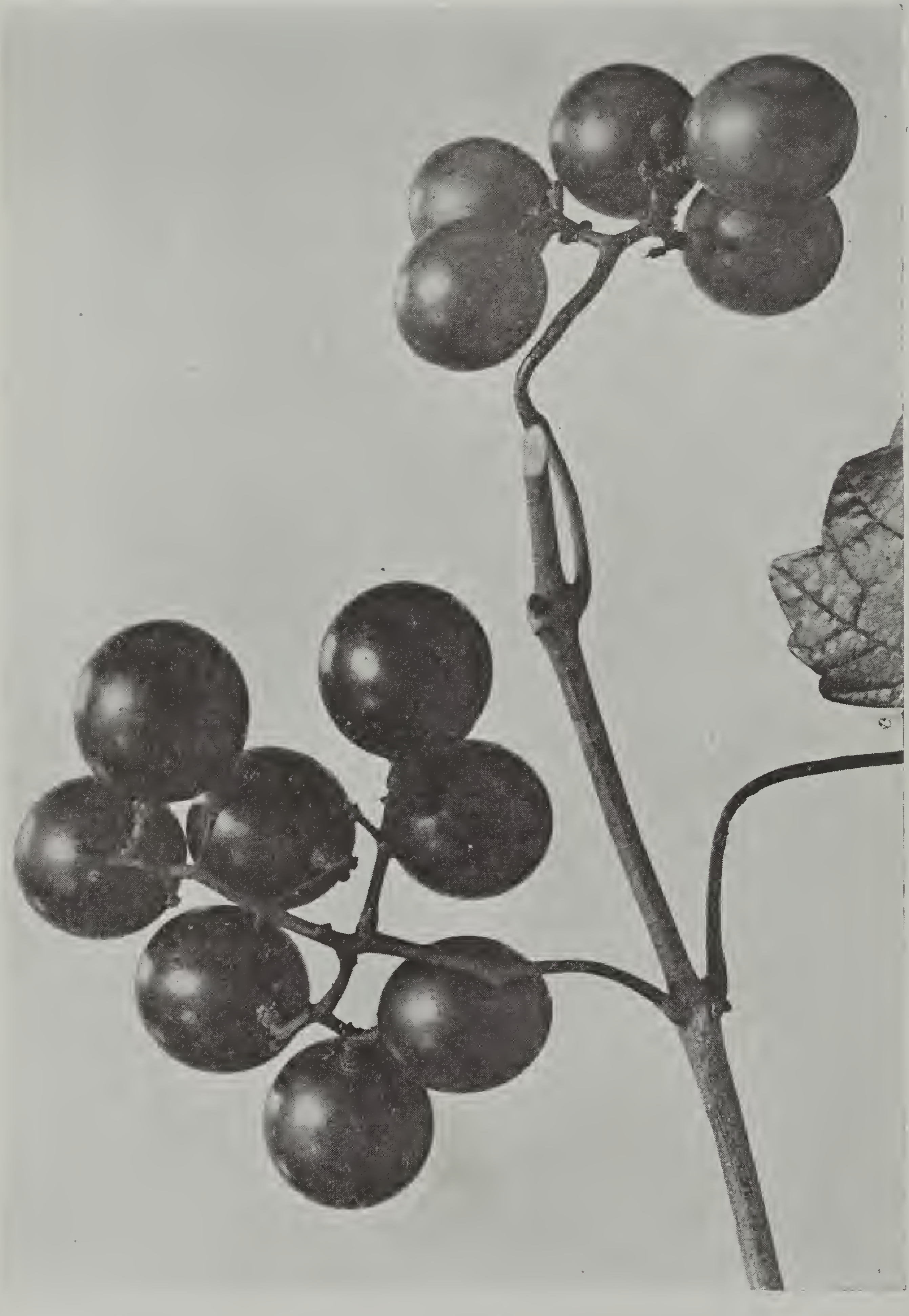

FIG. 6.—Two fruit clusters from the Hope vine whose flowers were protected from cross-pollination by having been inclosed in cloth bags. Natural size.

The layer that was transplanted to the vineyard grew well, and in 1912 ten branches were bagged before any buds had opened. Again, none of these flower clusters set fruit, and no fruit developed on the exposed branches of the vine.

In 1913 ten branches with a total of twenty flower clusters were carefully bagged, and this time eight of the ten bags contained some fruit. The whole vine had a fair sprinkling of fruit.

In 1914 twenty branches with not less than a total of forty clusters were bagged, and from each of these twenty bags some ripe fruit was harvested in the following September. The largest fruit cluster that was taken from these bags consisted of eight berries, while the largest cluster that was ever borne by the vine consisted of only ten berries.

In 1915 ten more branches with no less than twenty flower clusters were bagged, and fruit was harvested from eight of these bags in the fall of the year. Again there was a fair crop of fruit on the exposed parts of the vine, and every year since then a fairly good crop of fruit has been produced.

TABLE No. 1

A Test of Self-fertility of Hope Vine

Variety	Year	Number Branches Bagged	Number Bags Containing Fruit	Number Bags Containing No Fruit
Original vine, Hope	1910	2	0	2
	1911	35	0	35
	1912	10	0	10
Transplanted vine, Hope	1913	10	8	2
	1914	20	20	0
	1915	10	8	2

From the results of these bagging experiments we learn these important facts:

1. That the pollen of this vine, Hope, can under normal conditions fertilize the flowers on the same vine and on the same flower cluster.
2. That with proper cultivation and care this vine assumes the character of a hermaphrodite vine, while with neglect its pistils gradually cease to function and the vine assumes the general rôle of one that is staminate.
3. That cultivation, pruning, and general care exert a marked effect on the size and the regularity of the crop.

This last point is of considerable economic importance, because the general belief is that varieties of rotundifolia grapes can endure considerable neglect and still produce good crops.

IV. INVESTIGATIONS REGARDING THE INHERITANCE OF TYPES OF FLOWERS IN VITIS ROTUNDIFOLIA

With the discovery of the variety, Hope, we find that Vitis rotundifolia produces three distinct types of flowers instead of two. The important question now arises as to how and in what manner these three types of flowers are transmitted to the progeny of the first generation. Hedrick and Anthony (3), from tabulations of work done with many seedlings of Euvitis, report the following ratios:

Upright stamens × upright stamens=4.3 upright: 1 reflexed
Reflexed stamens × reflexed stamens=1.2 upright: 1 reflexed
Reflexed stamens × upright stamens=1. upright: 1 reflexed
Upright stamens × reflexed stamens=?

Whether these ratios are also representative of the laws that govern the transmission of types of flowers in Vitis rotundifolia is a question deemed worthy of investigation.

The method of procedure naturally resolves itself into a study of the progeny of crosses between vines with different types of flowers. Since there are flowers of three distinct types, namely, staminate flowers with upright stamens, hermaphrodite flowers with upright stamens, and hermaphrodite flowers with reflexed stamens, we may expect to make five different combinations, as follows:

1. Hermaphrodite flowers with reflexed stamens × staminate flowers (with upright stamens).
2. Hermaphrodite flowers with reflexed stamens × hermaphrodite flowers with upright stamens.
3. Hermaphrodite flowers with upright stamens × hermaphrodite flowers with upright stamens.
4. Hermaphrodite flowers with reflexed stamens × hermaphrodite flowers with reflexed stamens.
5. Hermaphrodite flowers with upright stamens × staminate flowers (with upright stamens).

The types of flowers that were observed in the progeny resulting from the first cross (hermaphrodite flowers with reflexed stamens × staminate flowers with upright stamens) has been partially discussed in Tech. Bul. No. 10 of this Station (5). Since that time more extensive and important data has been collected.

The following table shows the results so far obtained:

TABLE No. 2

SEX RATIOS AMONG PROGENY WHEN HERMAPHRODITE FLOWERS WITH REFLEXED STAMENS ARE CROSSED WITH STAMINATE FLOWERS

Female Parent	Male Parent	Number of Vines Staminate	Number of Vines Hermaphrodite With Reflexed Stamens	Number of Vines Hermaphrodite With Upright Stamens
Scuppernong	Light Male, No. 1	11	8	0
Scuppernong	Dark Male, No. 1	329	307	0
Scuppernong	Light Male, No. 2	507	494	0
James	Light Male, No. 2	59	64	0
Thomas	Light Male, No. 2	30	33	0
James	Light Male, No. 2	169	182	0
James	Light Male, No. 1	42	36	0
James	Light Male, No. 2	48	39	0
Flowers	Light Male, No. 2	103	82	0
Thomas	Light Male, No. 2	51	38	0
Scuppernong	Dark Male, No. 3	20	25	0
James	Dark Male, No. 3	9	6	0
James	Dark Male, No. 3	21	18	0
Thomas	Dark Male, No. 3	22	17	0
Latham	Light Male, No. 2	3	2	0
Thomas	Dark Male, No. 3	18	10	0
Thomas	Light Male, No. 2	15	14	0
Memory	Light Male, No. 2	5	6	0
Mish	Light Male, No. 2	2	2	0
Mish	Dark Male, No. 3	1	1	0
James	Light Male, No. 2	1	0	0
James	Light Male, No. 2	0	1	0
Scuppernong	Light Male, No. 2	4	2	0
Scuppernong	Light Male, No. 2	4	2	0
Seedling	Light Male	35	23	0
Totals		1,509	1,420	0

From the above table we learn two things: (1) That whenever hermaphrodite flowers with reflexed stamens are crossed with staminate flowers, only two tpes of flowers are observed in the progeny, namely, staminate flowers and hermaphrodite flowers with reflexed stamens (Plate I, Fig. 1, and Plate 2, Figs. 1-3). Not one seedling out of 2,929, representing the progeny of such a cross, has produced hermaphrodite flowers with upright stamens.* (2) That the ratio of the staminate flowers to the hermaphrodite flowers with the reflexed stamens is as 1509 :1420 or 105 : 1.

The second point under consideration is a study of the progeny resulting from hermaphrodite flowers with reflexed stamens when crossed with hermaphrodite flowers with upright stamens.

In 1911 five branches of a Scuppernong vine were bagged and later cross-pollinated with pollen from Hope as the male parent. In the following autumn a normal crop of fruit was gathered from these bags and a total of 108 seeds were harvested.

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*Husmann and Dearing (4) report the finding of two fruit-bearing seedlings with upright stamens among the progeny of vines which bear the reflexed stamens when crossed with staminate vines.

In 1912 the following similar crosses were made: Scuppernong × Hope, Thomas × Hope, and James × Hope. In each case the Hope vine was used as the staminate parent of the cross, and every cross seemed to be a congenial one, since a normal crop of fruit was harvested from these bags in the fall. A total of 1,351 seeds were obtained from the cross between Scuppernong and Hope, 1,117 seeds from the cross between Thomas and Hope, and 333 seeds from the cross between James and Hope.

In 1913 the variety Flowers was crossed with pollen from the Hope vine, and in all 309 seeds were harvested.

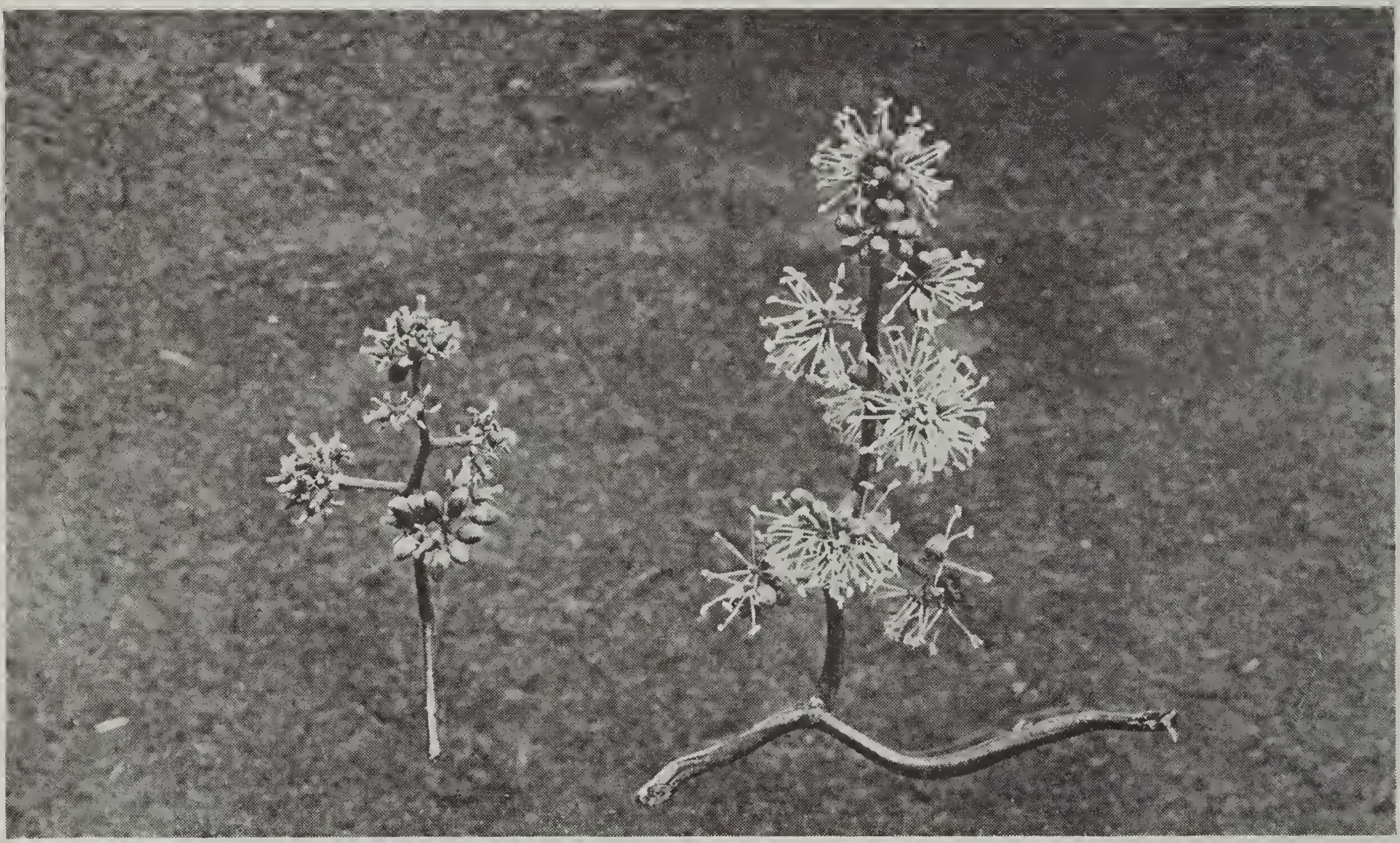

FIG. 7.—A flower cluster with reflexed stamens from a hermaphrodite vine and a flower cluster from a staminate vine. Note the comparative sizes and the characteristic type of each. Natural size.

In 1914 a light-colored seedling of James (F-13) was crossed with Hope and 149 seeds obtained. In the same year a dark seedling of Scuppernong (P-48) was crossed with pollen from Hope and 216 seeds obtained. All of these seeds were planted, and the resulting seedlings were later transplanted to nursery rows for study.

The following table gives the results obtained to date as to the different types of flowers that were observed among the progeny of the aforementioned crosses:

From this table we learn (1) that in every cross where Hope was used as the male parent on vines with reflexed stamens, only hermaphrodite vines have been obtained. Not one staminate vine has as yet appeared in any of these lots of seedlings.

TABLE No. 3.

SEX RATIOS AMONG THE PROGENY OF HERMAPHRODITE VINES WITH REFLEXED STAMENS CROSSED WITH HERMAPHRODITE VINES WITH UPRIGHT STAMENS

Year	Female Parent	Male Parent	Number of Seedlings Surviving	Staminate Flowers	Hermaphrodite Flowers Upright Stamens	Hermaphrodite Flowers Reflexed Stamens	Sex Not Determined
1911	Scuppernong	Hope	57	0	24	33	0
1912	Scuppernong	Hope	661	0	324	328	9
1912	Thomas	Hope	444	0	197	228	19
1912	James	Hope	158	0	79	74	5
1913	Flowers	Hope	104	0	39	53	12
	Totals		1,424	0	663	716	45

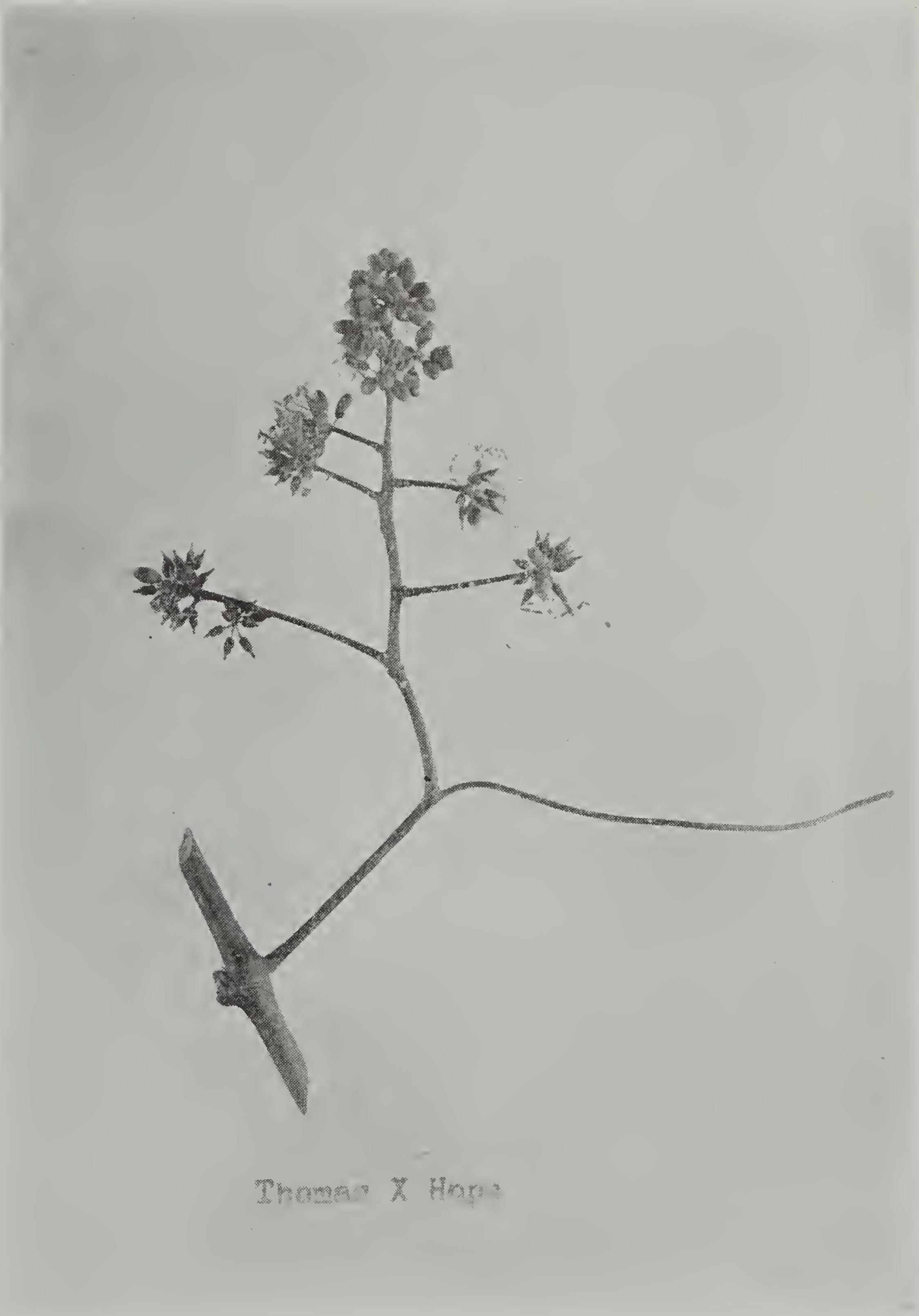

FIG. 8.—A typical hermaphrodite flower cluster with upright stamens. F_1 generation seedling of Thomas × Hope (three-year-old vine). Natural size.

By glancing at the record of the first lot, Scuppernong × Hope, the seeds of which were collected in 1911, we learn that of the 57 surviving vines, all are hermaphrodite in character and none are staminate.

In the second lot, where Scuppernong was again crossed with Hope, and seeds gathered in 1912, we see a repetition of the results that were obtained in the first lot, but in this case a much larger number of vines was involved. In the third, fourth, and fifth lots, in which Thomas, James, and Flowers were used to make similar crosses, the final results are practically the same. If we take the total of the numbers that were involved, we find that out of 1,424 vines, 1,379 produced hermaphrodite flowers, not one produced staminate flowers, and 45 were too weak to produce any flowers at all. These last mentioned vines unquestionably will also bear hermaphrodite flowers when mature.

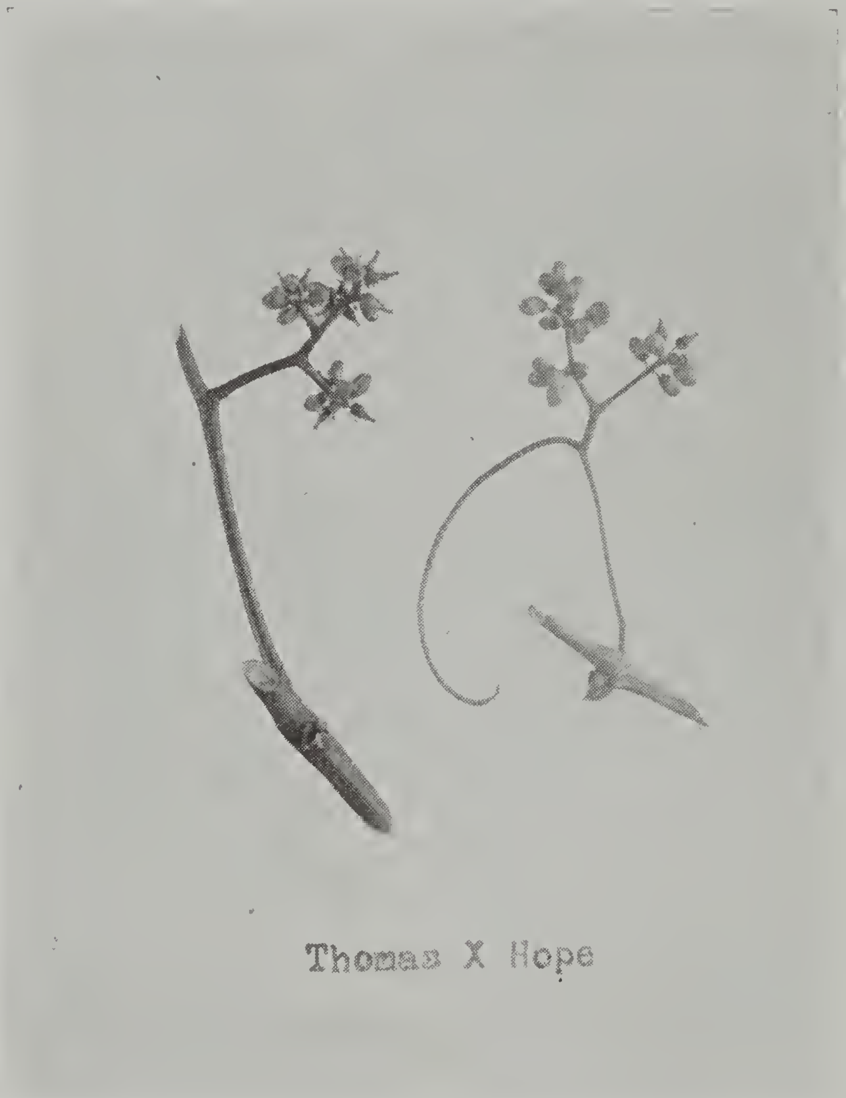

Fig. 9.—Two typical hermaphrodite flower clusters with reflexed stamens. F_1 generation seedling vine of Thomas × Hope (three-year-old vine). Natural size.

We also learn (2) that these seedling hermaphrodite vines are of two kinds (Plate 1, Figs. 3-6), those which produce flowers with upright stamens and those which produce flowers with reflexed stamens. The ratio between these two floral types is quite uniform, and appears to be about equally divided. By adding the total numbers, we find that the upright stamen type is to the reflexed stamen type as 663:716, or a ratio of 1:1.07.

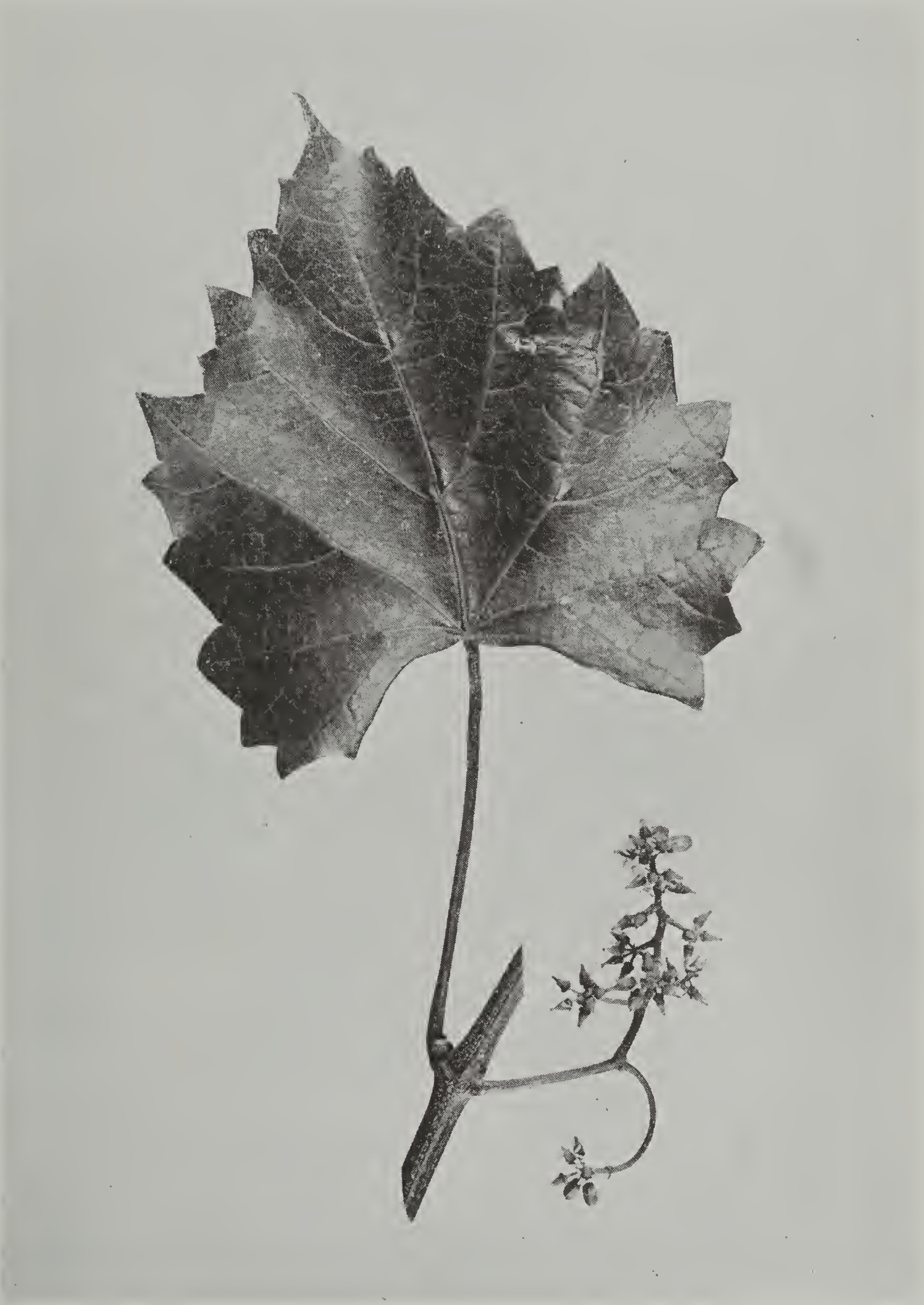

FIG. 10.—A typical flower cluster from the variety Flowers. Natural size.

Before drawing conclusions relative to the ratios existing between the different types of flowers, the utmost care should be exercised in getting as complete data as is possible, because very often hermaphrodite vines with the reflexed type of stamens are observed to require one or more years to reach full maturity than staminate, or hermaphrodite vines with upright stamens, This point is well illustrated in the following table.

TABLE No. 4

MISLEADING SEX RATIOS

Parentage	Age of Vines	Total Number Vines	Number Vines Blooming	Staminate Vines	Hermaphrodite Vines—Reflexed Stamens	Hermaphrodite Vines—Upright Stamens
Scuppernong × L. Male	2 years	51	18	16	2	0
Thomas × L. Male	2 years	72	31	23	8	0
James × L. Male	2 years	64	47	27	20	0
James × L. Male	2 years	63	31	17	14	0
Scuppernong × L. Male	2 years	128	66	55	11	0
Scuppernong × D. Male	2 years	644	198	143	55	0
Scuppernong × L. Male	2 years	832	176	157	19	0
Lab. Aest. × Lab. Male	2 years	65	19	19	0	0
Totals		1,919	586	457	129	0
Scuppernong × Hope	3 years	57	8	0	2	6
Scuppernong × Hope	2 years	661	79	0	18	61
Thomas × Hope	2 years	443	108	0	27	81
James × Hope	2 years	157	31	0	9	22
Total		1,318	226	0	56	170

From the first part of this table it becomes evident that the staminate vines in every case outnumber the hermaphrodite vines which bear the reflexed type of stamen, and that the ratio of the total number of such vines at the age of two years from seed is 457 staminate to 129 hermaphrodite vines with reflexed stamens, or 3.5 : 1. But there are still 1,333 vines which have not yet bloomed. It is necessary, therefore, that the types of flowers of all of these be considered before final conclusions are drawn.

The second part of the table shows the numbers of vines of the different types that bloomed at the age of two and three years when Hope was used as a male parent. Again, those plants that bear the upright stamens are in the majority, 170 vines with upright stamens as compared with 56 vines of the type that bears the reflexed stamens, or a ratio of 3 : 1.

Referring to Tables Nos. 2 and 3, we observe that when these same vines are three or more years of age the ratios of the vines that bear upright stamens to those that bear the reflexed stamens have been almost equaled. The ratios in both of these tables no longer stand as 3 : 1, but practically as 1 : 1, the theoretical ratio.

The third point under consideration deals with the ratio of the types of flowers that appear among seedling vines when both of the parent vines produce hermaphrodite flowers with upright stamens.

While testing the Hope vine for self-fertility, a certain number of seeds were secured from fruits that had developed in the cloth bags without the introduction of any foreign pollen. In 1913 sixty-eight seeds were thus secured and planted. From these 68 seeds only 7 plants were obtained, of which, at the present writing, only 6 are alive. In 1915 two of these vines bloomed and each produced only staminate flowers.

In 1915, 47 seedlings were obtained from the same mother vine, and in 1916, 530 more seedlings from a similar origin but from different vines, were set in the nursery row for observation and study.

In the fall of 1913 a large number of seeds were taken from fruits of the Hope vine which had developed under natural conditions, that is, with the flowers uncovered and subject to any cross-pollination that might take place. From these seeds 142 plants were obtained and some of these have bloomed this year (1916). The types and numbers of vines that have bloomed and been observed are as follows: staminate vines, 21; hermaphrodite vines with upright stamens, 31; hermaphrodite vines with reflexed stamens, 4. The number of vines not blooming was 86. Of these 86 non-blooming vines, it was confidently expected by the writer that 17 vines, by virtue of their size and age, would produce hermaphrodite flowers with reflexed stamens. The remaining 59 seedlings were too small and weak to produce any flowers. If we accept the numbers of these vines as stated, the ratios of the different types is 21:31:21. What the final ratio will be when all of the vines shall have bloomed becomes, at most, an interesting matter for speculation.

TABLE No. 5

THE EXTENT OF NATURAL CROSSING THAT MAY TAKE PLACE AMONG THE SELF-FERTILE SEEDLING VINES OF THE HOPE VINE PROGENY WHEN GROWING IN MASS

Lot	Color Mother Vines	Total Number Seedlings Grown	Number Seedlings Light Color	Number Seedlings Dark Color
1	Light	112	110	2
2	Light	64	58	6
3	Light	59	55	4
4	Light	16	13	3
5	Light	105	88	17
6	Light	20	19	1
7	Light	104	104	0
8	Light	40	38	2
9	Light	96	90	6
Totals		616	575	41

In this work, seeds from light-colored vines only were selected, because, as the factor for coloration is dominant to its absence, the appearance of dark-colored seedlings in any of these several lots would be strong evidence in favor of cross-pollination.

From the evidence presented in Table No. 5, we learn that in eight out of nine lots of seedlings some dark-colored plants have appeared. By way of explanation, it should be said that the dark-colored vines, 547 in number, standing adjacent to the light-colored ones from which the seeds were taken, were heterozygous for the light and the dark colors. If, then, any crossing took place between the light and the dark vines, the resultant dark-colored seedlings, according to the Mendelian laws, represent only about one-half of the actual number of such a cross. This very meager evidence gives us some idea of the actual amount of intercrossing that takes place among our strain of hermaphrodite vines that have the upright stamen. The ratios of colors that were obtained among these seedlings, however, are not to be taken as an index of the extent of cross-pollination that usually takes place among such seedling vines, but simply as an indication that some crossing has resulted.

The fourth point under consideration deals with the ratios between types of flowers among seedling vines when both of the parents bear reflexed stamens. Since Bul. No. 209 of this Station (1) discusses at some length the self-sterility and the inter-sterility of all rotundifolia grape vines which bear reflexed type of stamen, we understand immediately why such seedling vines have not been produced and, hence, why we cannot establish a ratio between types of flowers resulting from such a cross.

The fifth point under consideration deals with the ratios between types of flowers in the progeny when hermaphrodite flowers with upright stamens are crossed with staminate flowers. In 1915 twenty flower clusters of this Hope vine were carefully emasculated and bagged and later, after about twenty-four hours, viable pollen from a staminate vine was brushed over the pistils. No fruits developed in any of these bags.

The same spring 53 clusters on one of its self-fertile seedling vines were carefully emasculated and, when the pistils became receptive, were pollinated with fresh, viable pollen from a staminate vine. Not one of these flower clusters, however, has produced so much as a berry. At the same time, two large flower clusters on this same self-fertile vine, the flowers of which were not emasculated, were dusted with viable pollen from a staminate vine which is known to be homozygous for dark color. Eighteen berries ripened and a total of 61 seeds were obtained. These seeds were planted in 1916 and from them 59 seedlings were grown. Each of these 59 seedlings were light in color and not one was dark. This indicates that the seedlings were not the result of a cross between two separate vines, but the product from self-fertilized flowers on the mother vine, since dark color of vine is dominant over light color. [Reimer and Detjen (5), Hedrick and Anthony (3)].

In 1916 flowers of twelve more clusters from light-colored self-fertile seedlings were carefully emasculated and later hand pollinated with viable pollen from another staminate vine which is also known to be homozygous for dark color. Not one of these flower clusters produced a berry. At the same time the flowers on 50 or more clusters on similar light-colored, self-fertile seedling vines (all seedlings of Scuppernong and Hope) were emasculated, although less carefully in regard to the selfing of the flowers, and immediately cross-pollinated with pollen from a staminate vine which was known to be homozygous for dark color.

Of these 50 bags, 38 were later removed because they contained no fruits. Twelve bags contained one or more fruits, and from these 142 seeds were secured. These seeds will be duly planted, and the colors of the resulting seedlings were well watched with keen interest.*

V. CORRELATION BETWEEN TYPE OF STAMEN AND CHARACTER OF POLLEN AND BETWEEN SIZE OF FLOWER CLUSTER AND CHARACTER OF POLLEN.

The theory that the reflexed type of stamen in Vitis is usually associated with degenerate, impotent pollen, and with complete or nearly complete self-sterility, is generally conceded by most of the present-day investigators. In like manner the corollary of this theory, that upright stamens are generally an index of normal viable pollen and self-fertility, is also recognized [Dorsey (2), Hedrick and Anthony (3), and Beach (6)].

While this correlation between type of stamen and character of pollen holds quite true (Plates III and IV), the numerical relations that exist between viable and defective pollen grains in the same flower seem to be more or less variable.

Booth (7) says, in effect, that among garden varieties of grapes one can find, with the aid of a microscope, a whole series that produce more or less defective pollen. The percentage of this defective pollen in such varieties ranges all the way from almost none in the self-fertile varieties to 100 per cent in those that are self-sterile. What relation the amount of this defective pollen bears to the type of stamen by which it was produced, he does not make clear.

Hedrick and Anthony (3) have successfully crossed cultivated or garden varieties of *Euvitis* where both of the parents bore the reflexed type of stamen. The same authors have grown numbers of seedlings from such varieties when close pollinated, all of which goes to show that some normal pollen grains are frequently and habitually produced by some varieties that have the reflexed type of stamen.

While such varieties of grapes produce some viable pollen grains, not enough of these are formed, however, to class such vines among the perfectly self-fertile varieties. Barring the effects of ravaging diseases and other external factors, the looseness of a fruit cluster frequently is an indicator of the production of an insufficient amount of viable pollen for perfect fertilization. Most of the varieties with the reflexed type of stamen, however, are completely self-sterile, a fact which is due to their defective and degenerate pollen. This is probably more true of wild vines of the native species than of most garden varieties which are representative of hybridization and selection. Booth (7) says: "In the wild, the grape is diœcious, and fertile pollen borne by the pistillate flower with reflexed stamens is rare, if it occurs at all."

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*Since the manuscript of this bulletin was prepared these 142 seeds have been planted and 39 seedlings have appeared above ground. Of these, 25 are dark in color and 14 are green. The dark seedlings, therefore, are the result of a cross between the light-colored and perfect flowered vine with upright stamens and a dark-colored staminate vine. Later on, these dark seedlings will be transplanted to the nursery row, and when grown the types of their flowers will be duly recorded.

Among the many vines of Vitis rotundifolia, both the cultivated and the wild, that have been studied at this Station, not one case has been discovered where normal fruit is definitely known to have developed from pollen that was produced by the reflexed type of stamen.*

The production of seedless berries must naturally be omitted from the present discussion, because it is not the result of a sexual union between two normal gametes, and it is not the purpose of this bulletin to discuss parthenocarpy in relation to the pollination of flowers.

That all vines of Vitis rotundifolia which bear the reflexed type of stamen are completely self-sterile is borne out by the published results in Bul. No. 209 (1). To these results we can add from Tech. Bul. No. 10 (5), that when 693 seedlings were raised from Scuppernong crossed by a male vine homozygous for dark color, not one light-colored vine appeared in the whole lot. It will be remembered that in crossing varieties of rotundifolia grapes which have the reflexed type of stamen, the usual emasculation process is habitually omitted, because if any fertile pollen were produced, its presence could quickly be detected in the progeny of such a cross by the presence of light-colored seedlings. Likewise, the 62 seedlings from James crossed with the same dark male vine were all dark in color, notwithstanding the heterozygous nature of the James variety with respect to light and dark colors.

The correlation of upright stamens with viable pollen and self-fertility of the grape is also generally recognized. While the introduction of rotundifolia grape vines that bear upright stamens is of recent date [Reimer and Detjen (1), and Husmann and Dearing (4)], all have proved to be self-fertile, at least in varying degrees, and consistent with the small fruit clusters of this type of grape. Hope, our original wild vine, and its vegetative offspring, in some years under favorable conditions, has proven to be self-fertile, as recorded elsewhere in this bulletin.

All of the perfect flowered seedlings of this same vine, the founder of our race of self-fertile rotundifolia grape vines, also produce normal viable pollen like their parent and are self-fertile in the same degree.

Husmann and Dearing (4) report that both of their original seedlings with upright stamens are fruitful and self-fertile. Also, that all of their more recent seedlings of similar character are likewise self-fertile.

In 1915 flower clusters on a number of perfect flowered seedling vines were bagged for a test of their self-fertility. The bags happened to be distributed as follows: one bag on each of 64 different vines, six bags on one special vine, and eighteen on another special vine. The results obtained are presented in the following table.

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*The two berries that are mentioned in Bul. 209 of this Station as having developed under closed cloth bags on a Scuppernong vine are regarded, in the light of more recent investigation, as the result of cross-pollination, which must have taken place previously to the bagging operation. That a single flower in the bag which covered several hundred similar flowers should receive sufficient pollen to produce three or four seeds, as was found in this case, while other flowers under the same bag by laws of chance should receive absolutely none, appears almost incredible. It is much easier to conceive of two fertilized blooms having been overlooked in the examination and preparation of the many thousands of buds that were covered in the experiment.

TABLE No. 6

SELF-FERTILITY OF HERMAPHRODITE SEEDLING VINES.

Parentage of Seedling Vine	Number Vines Represented	Number Bags Used	Character Stamens	Number Bags Containing Fruit	Number Bags Without Fruit
James × Hope	20	20	Upright	11	9
Scuppernong × Hope	24	24	Upright	14	10
Thomas × Hope	20	20	Upright	9	11
Totals	64	64	Upright	34	30
Scuppernong × Hope	1	18	Upright	16	2
Thomas × Hope	1	6	Upright	5	1

In 1916 eight special vines were tested for self-fertility by placing ten bags at random on unopened flower clusters of each. The results that were obtained in this test are set forth in the following table:

TABLE No. 7.

SELF-FERTILITY OF HERMAPHRODITE SEEDLING VINES

Parentage	Number Branches Bagged	Character Stamens	Number Bags Containing Fruit	Number Bags Without Fruit
Flowers × Hope	10	Upright	0	10
Flowers × Hope	10	Upright	6	4
Flowers × Hope	10	Upright	3	7
Flowers × Hope	10	Upright	8	2
Thomas × Hope	10	Upright	0	10
Thomas × Hope	10	Upright	6	4
Thomas × Hope	10	Upright	0	10
Scuppernong × Hope	10	Upright	5	5
Total	80	Upright	28	52

In the two preceding tables we observe at a glance that in many cases fruit was produced and obtained without the aid of any cross-pollination. No such results have ever before been observed or obtained when flowers with reflexed stamens were isolated from chances of cross-pollination.

It will be observed, however, that not all of the vines which were included in this test produced fruits in the bags. This, however, is not a strange phenomenon when we consider the fact that each one of the sixty-four vines was represented by the flowers of only one or two clusters contained in only one bag. Even the eight vines on which ten and more clusters were bagged gave evidence that at times no fruits may set in some of the bags, and the vines still show signs of self-fertility by reason of the fruits that developed in the others. The failure of one or all clusters to produce fruit cannot well be regarded as proof of absolute sterility. Any one at all familiar with the rotundifolia species knows how common it is for young, vigorous vines to shed a great many of the flower clusters without setting fruit when heavily pruned and crowded together as are these vines.

The point is well illustrated in the last table, where all of the eight vines shed some of their flower clusters, and three out of the eight produced no fruits at all inside of the bags. As the season of 1915 was an exceptionally unfavorable one for fruit production in the experimental vineyard, these negative results must be looked upon as indicative only, but a further trial or investigation doubtless will prove these same vines to be self-fertile like the others. All of the vines that set no fruit in the bags were subsequently examined as to the character of their pollen, and in no case did this prove to be anything but normal.

From these results we conclude that upright stamens in rotundifolia grape flowers are always associated with viable pollen, and, if the pistils are normally developed, with a certain degree of self-fertility of the vine.

It might be of interest to know that along with the character of the pollen, and the type of stamens, there is correlated a third character, namely, that of size of flower cluster. The staminate vines produce comparatively large flower clusters; the hermaphrodite vines that bear upright stamens produce flower clusters of a similar size, while the hermaphrodite vines with the reflexed stamens generally produce small flower clusters.

Notwithstanding the fact that the flower clusters of the three different types of vines vary considerably among themselves, it is always possible to determine with comparative ease and accuracy what the type of stamen and the character of pollen will be while the vine is still in bud.

When hermaphrodite vines that bear the reflexed type of stamen are crossed with staminate vines, the resulting progeny can be separated and grouped according to their sexes by means of the size of their respective flower clusters. In like manner the progeny of hermaphrodite vines that bear the reflexed type of stamen, when crossed with pollen from hermaphrodite vines that bear the upright stamens, can be separated and grouped according to the character of their pollen by nothing more than their characteristic flower clusters.

These correlated characters are of considerable importance to the rotundifolia grape breeder, because by means of them a comparatively early selection and segregation of vines can be made with respect to normal viable pollen and self-fertility.

VI. SIMILARITY BETWEEN FLOWER CLUSTERS THAT ARE BORNE ON HERMAPHRODITE VINES WITH UPRIGHT STAMENS AND THOSE THAT ARE BORNE ON STAMINATE VINES.

As the staminate vines when in bud, and before any flowers have opened, can be readily distinguished from the imperfect hermaphrodite vines by the difference in the size and shape of their respective flower clusters (see Fig. 7), so in like manner the perfect hermaphrodite vines

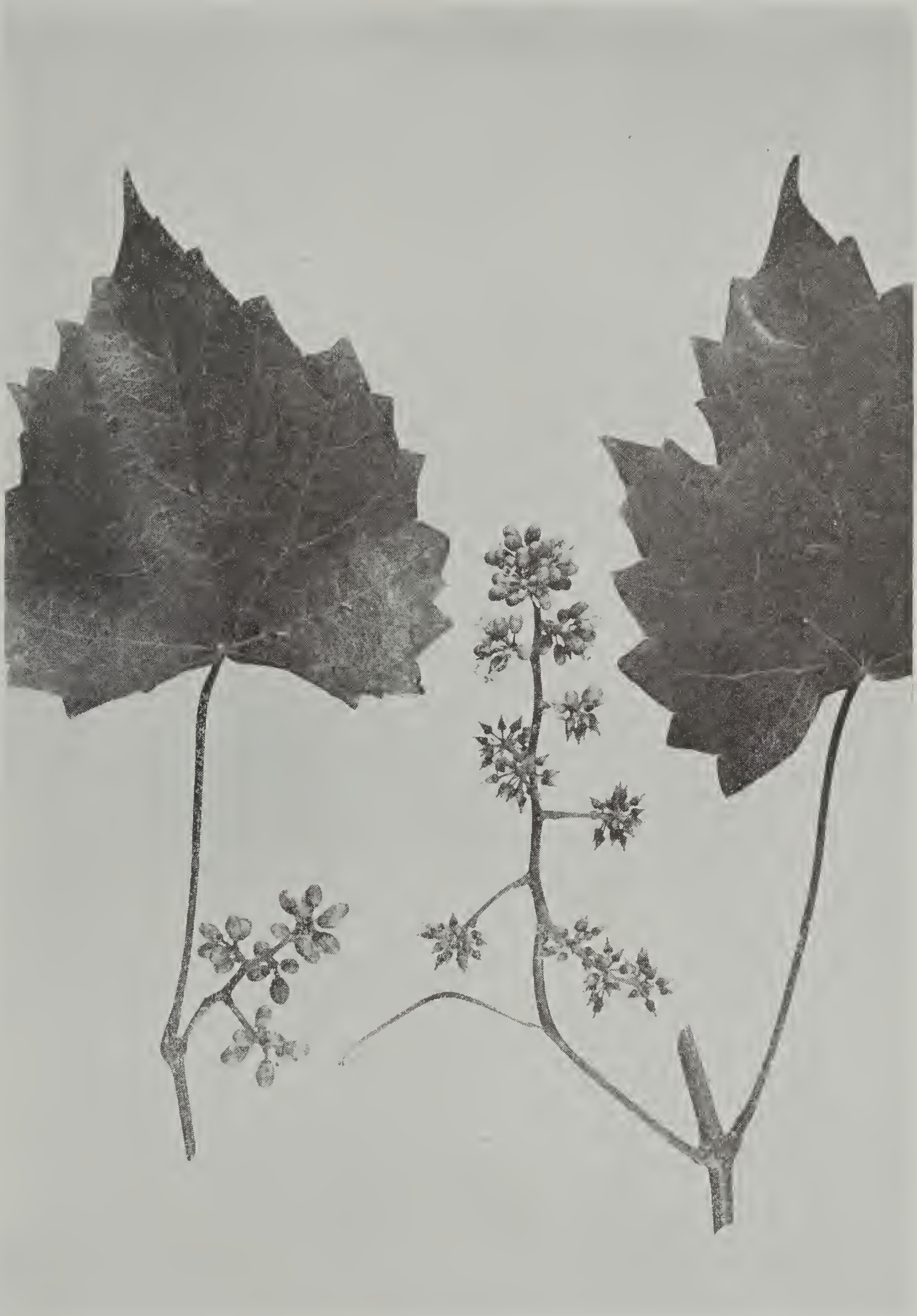

Fig. 11.—Two flower clusters from hermaphrodite vines of the F_1 generation of Flowers × Hope. The cluster on the right bears the upright type of stamen, while the cluster on the left bears the reflexed type of stamen. These two clusters are the largest of their kind that could be found among 103 vines. Natural size.

can be distinguished from the imperfect hermaphrodite vines (see Figs. 8, 9, 10, and 11). But a staminate vine and a perfect hermaphrodite vine when in bud cannot be so easily distinguished from one another, if at all.

A flower cluster on a perfect hermaphrodite vine, like one on a staminate vine, normally consists of a great many flowers crowded together on a more or less lengthened rachis (see Figs. 7, 8, 11, and 12). The largest number of buds that were ever counted in a single flower cluster on a staminate vine was 384 and on a perfect hermaphrodite vine (Hope) 299. This staminate vine, however, is one of the best ever observed by the writer among wild vines, while Hope is the first of its kind that was ever recorded among rotundifolia grape vines.

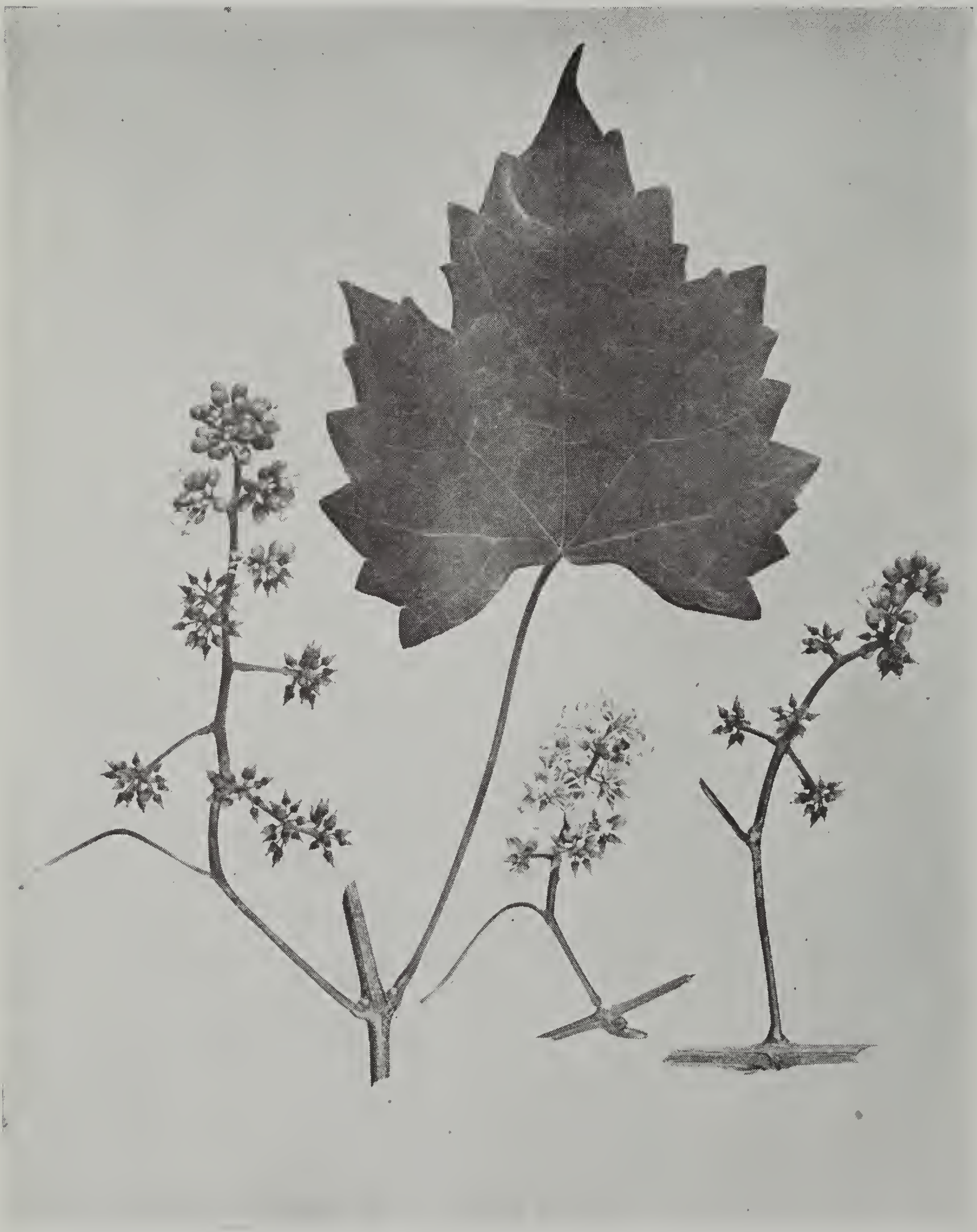

FIG. 12.—Variations in size of the flower clusters from hermaphrodite vines that bear the upright type of stamen. Natural size.

When perfect hermaphrodite vines become as plentiful as the staminate vines, then by virtue of a wider choice we can expect to find flower clusters on such vines equally as large as those on any staminate vine. Selected perfect hermaphrodite seedlings now in our possession doubtless will produce flower clusters equally as large as those that were observed on the staminate vine, but the average flower clusters on the average perfect hermaphrodite vines are about the same in size as those on similar staminate vines.

VII. The Probable Origin of the Hope Vine

By comparing descriptions of Hope with that of any staminate vine, one can readily see that the two have many things in common. Both vines produce pollen which, when mixed, cannot, by aid of the microscope alone, be distinguished as being the product from two different vines (Plate III, Figs. 1 and 3, and Plate IV, Figs. 1, 2, and 3); the pollen of both is viable and will germinate in sugar solutions; both produce upright stamens of identical size, shape, and appearance (see Figs. 3 and 7); and both produce large flower clusters typical of the staminate vines. The only point of difference lies in the fact that the Hope flowers possess functioning pistils which are not found in any staminate vine.

The seedlings of any hermaphrodite variety that has the reflexed type of stamen, when crossed with Hope as the male parent, are always hermaphrodite vines, but of two kinds: (1) those that bear the upright stamens, and (2) those that bear the reflexed stamens (see Figs. 8 and 9). These types occur in the expected ratio of 1:1, just as those whose male parent is a normal staminate vine. These perfect flowered seedling vines with upright stamens also come to maturity earlier than do those with the reflexed stamens, just as do normal staminate seedlings (see Table No. 4). The whole appearance and behavior of the Hope vine, and of its perfect flowered seedlings, with the exception of the regenerated and functioning pistils (Plate I, Figs. 2, 4, and 6), is that of a normal staminate vine.

Again, some of the flowers on the Hope vine and some on its perfect flowered progeny have been observed to be more or less intermediate between perfect hermaphrodite flowers and staminate flowers (Plate II, Figs. 4-9). Some of the pistils were dwarfed, some rudimentary, and some few were suppressed entirely (see Fig. 14), as is the case of staminate flowers. Such flowers were found to occur more abundantly on some vines than on others, but usually in all cases they were found on the lower and more poorly nourished branches of the vine.

All of this evidence points strongly toward the assumption that this Hope vine was primarily a staminate vine, with a staminate constitution, but one which from some unknown cause has undergone a partial regeneration of its pistils, some of which are able to function.

When flowers on many staminate vines are examined, it will be observed that there exists much variation in the size of the suppressed

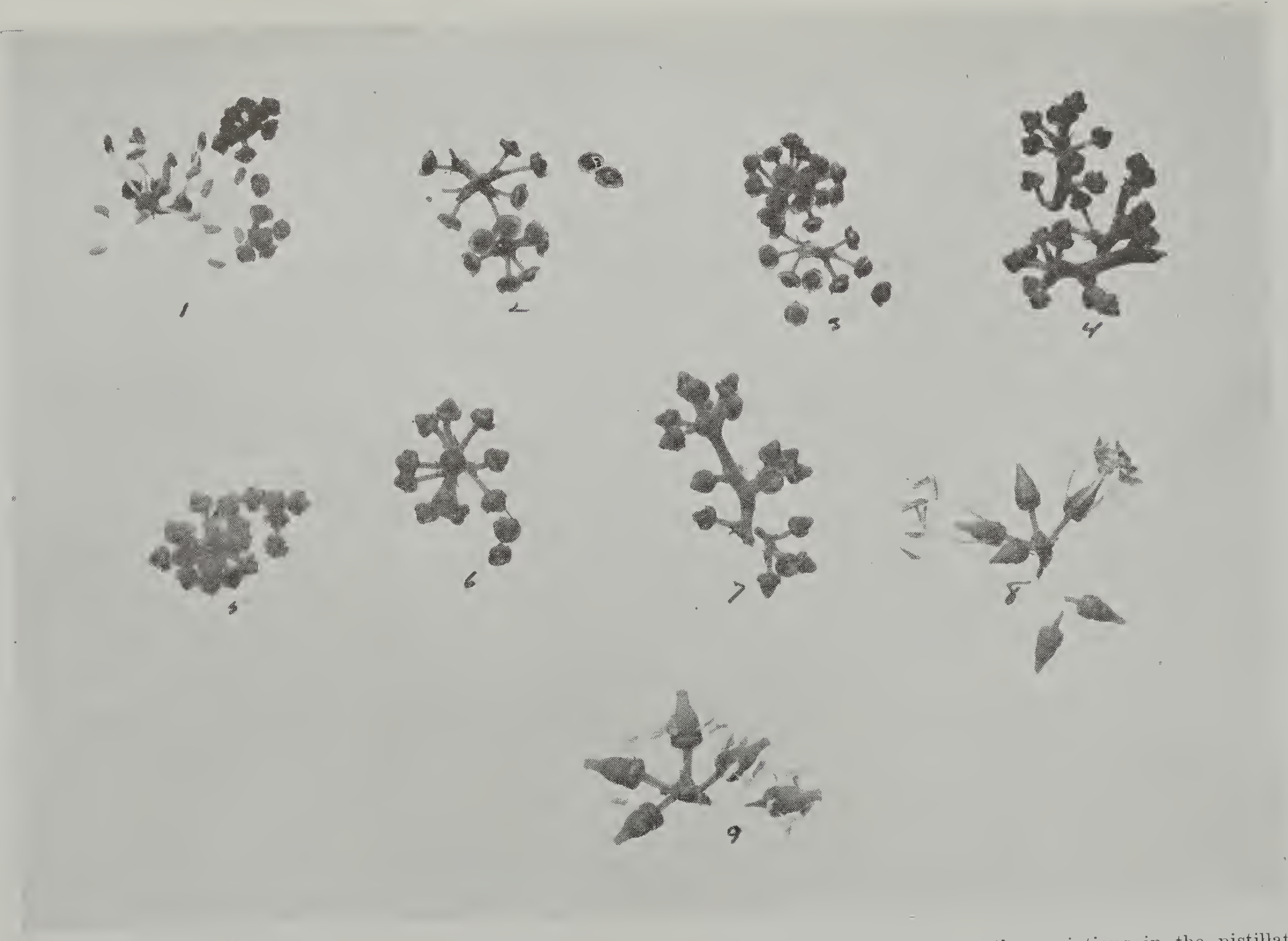

Fig. 13.—Variation in the pistils of Vitis rotundifolia. Numbers 1 to 7, inclusive, represent the variations in the pistillate rudiments which were observed on different staminate vines. No. 8 shows the type of pistil that is found on hermaphrodite vines that bear the upright stamen. No. 9 shows the type of pistil that is found on hermaphrodite vines that bear the reflexed stamen. Natural size.

pistils (see Fig. 13, and Plate II, Figs. 1-3). In fact, many pistillate rudiments in flowers on staminate vines are larger than many similar rudiments in flowers on hermaphrodite vines. With this variation of the pistils in mind, it is not difficult to imagine that by a sudden release from an inhibiting factor these pistils, so long suppressed, have suddenly grown out and become functional.

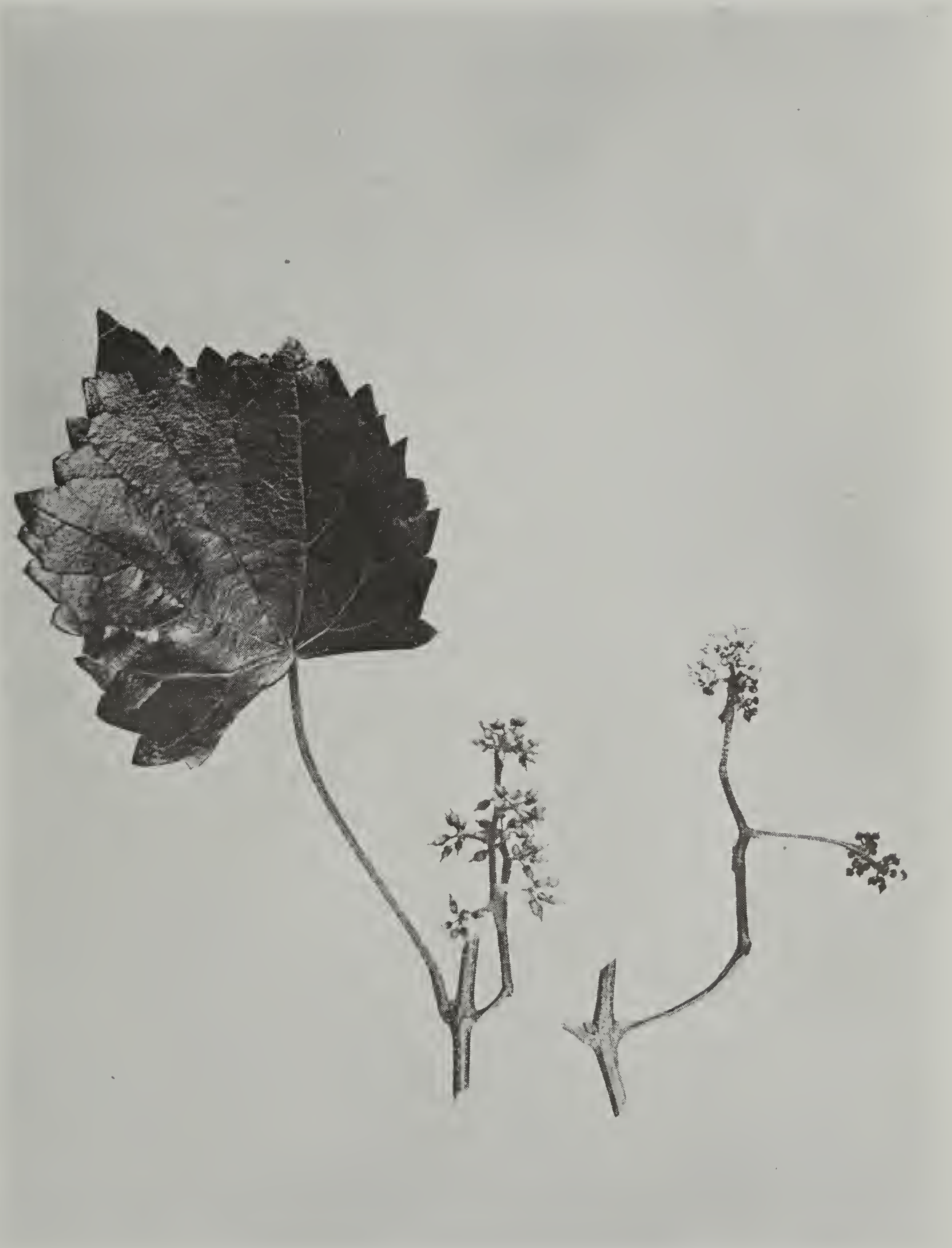

Fig. 14.—Variations in size of the pistils frequently found on the hermaphrodite seedlings of Hope which bear the upright stamens. Cluster on the left represents the normal type of pistil, while cluster on the right shows considerable reduction in size of the pistils. Natural size.

The strongest link in the chain of evidence yet secured, which points out that the Hope vine is essentially a staminate vine with its suppressed pistils regenerated, lies in the evidence already submitted, to the effect that although fertile when treated with pollen from the same or similar flowers, these pistils are often unfertile when treated with pollen from a staminate vine.

Whether our race of self-fertile hermaphrodite vines represents a progressive step in the line of the evolution of rotundifolia grape flowers is a question that is not so easily proven. The fact that staminate vines show much variation in the size of the suppressed pistils, and that all of the hermaphrodite vines which function as pistillate vines bear stamens and pollen, although functionless, seems to indicate that early in the history of Vitis all of the vines bore hermaphrodite flowers whose stamens and pistils were perfect and functional. Considering the facts of the case in the light of our recent investigations, we may assume our original vine Hope to be a partial reversion to its ancient hermaphrodite ancestors.

VIII. SUMMARY

The investigations regarding the inheritance of self-fertility in Vitis rotundifolia which are being carried on at this Station have led to the following conclusions:

1. That Hope, the first discovered hermaphrodite grape vine of the species Vitis rotundifolia, which bears upright stamens, is self-fertile.

2. That the self-fertility of the Hope vine is variable, and seems to depend on its inner constitution.

3. That floral types in Vitis rotundifolia are transmitted to the progeny in definite ratios. Substituting S for staminate flowers, R for hermaphrodite flowers with reflexed stamens, and U for hermaphrodite flowers with upright stamens, we find that

$$\begin{aligned} R \times S &= 1\,R : 1.06\,S \\ R \times U &= 1.07\,R : 1\,U \\ U \times U &= S : R \text{ (ratios not determined)} \end{aligned}$$

4. That the Hope vine, and some of its self-fertile progeny, apparently will not cross with staminate vines.

5. That in Vitis rotundifolia the upright stamen in hermaphrodite flowers is correlated with normal, viable pollen, and self-fertility; that the reflexed stamen in hermaphrodite flowers is always associated with defective pollen and self-sterility.

6. That the Hope vine and all of its seedlings, which bear upright stamens, appear and behave essentially like staminate vines except for the facts that the flowers usually contain well developed pistils, and that fruit is subsequently produced.

7. That the Hope vine probably is a staminate vine whose long suppressed pistils have suddenly been regenerated and have recovered the power to function.

8. That the prototype of our present-day rotundifolia vines probably was a true and functioning hermaphrodite.

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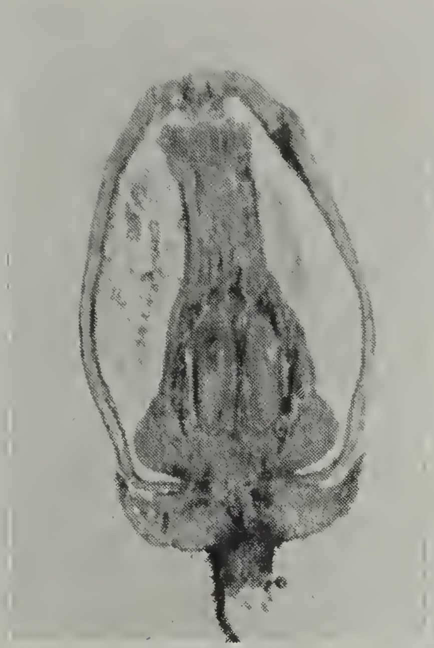
PLATE I

Photomicrographs of median sections of flower buds of Vitis rotundifolia

Fig. 1. Scuppernong
Fig. 2. Hope
Fig. 3. Hermaphrodite seedling with reflexed stamens
F_1 generation of Scuppernong × Hope
Fig. 4. Hermaphrodite seedling with upright stamens
F_1 generation of Scuppernong × Hope
Fig. 5. Hermaphrodite seedling with reflexed stamens
F_1 generation of Thomas × Hope
Fig. 6. Hermaphrodite seedling with upright stamens
F_1 generation of Thomas × Hope

PLATE I



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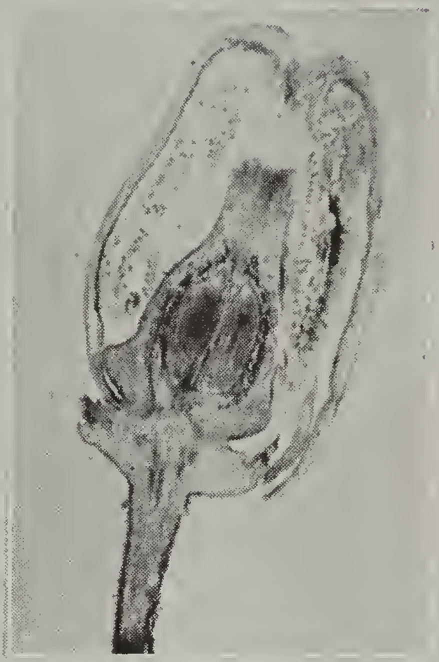

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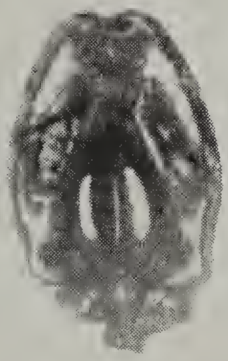

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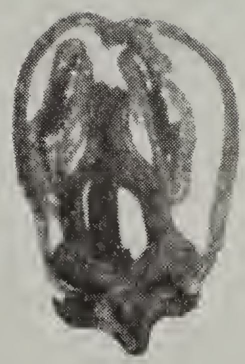

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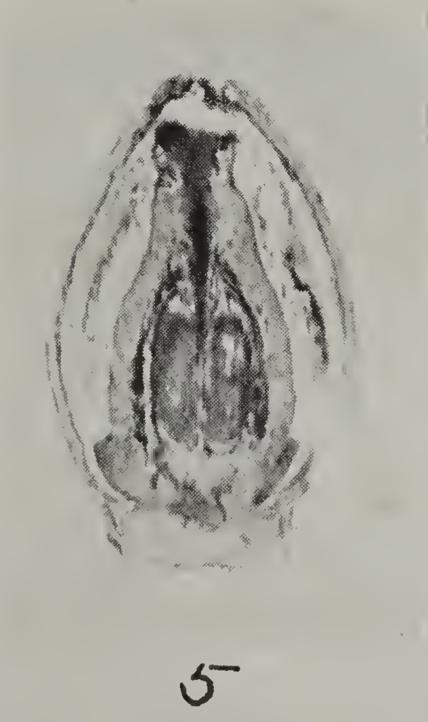

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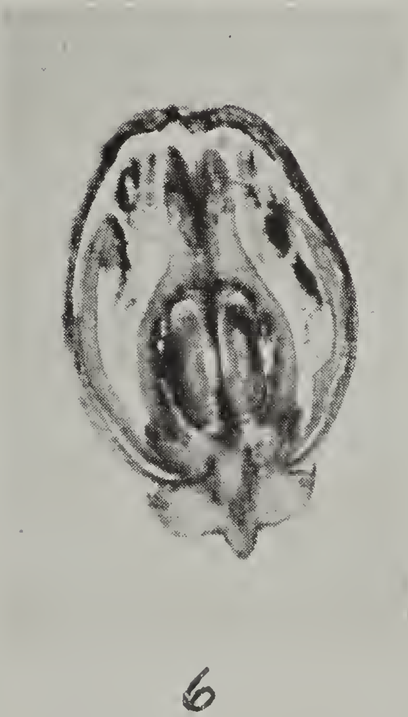

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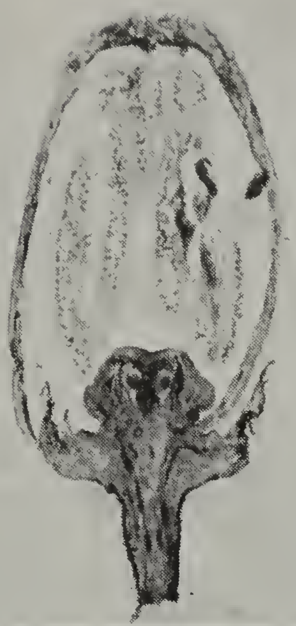
PLATE II

Photomicrographs of median sections of flower buds of Vitis rotundifolia

FIG. 1. Staminate vine (S-10)
FIG. 2. Staminate vine (Light, No. 1)
FIG. 3. Staminate vine (D-21) showing rudiment of a style
FIG. 4-7. Hope, variations in the development of the pistils
FIG. 8. Pistillate rudiment found with some flowers on hermaphrodite vines that bear upright stamens, F_1 generation of Thomas × Hope
FIG. 9. Pistillate rudiment found with some flowers on hermaphrodite vines that bear the upright stamens, F_1 generation of Scuppernong × Hope

PLATE II



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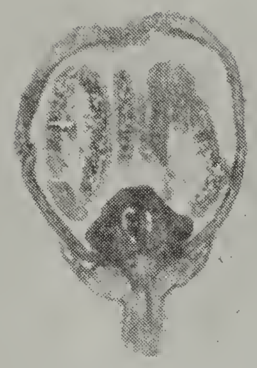

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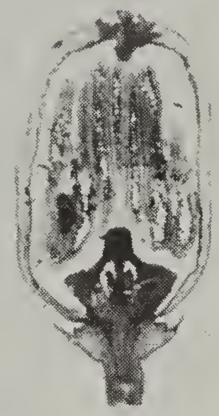

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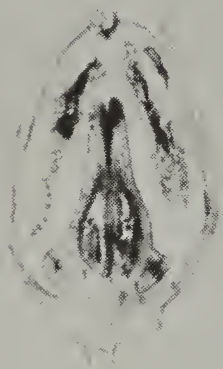

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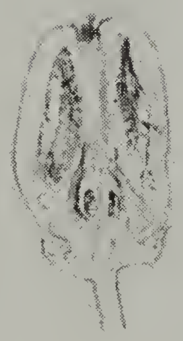

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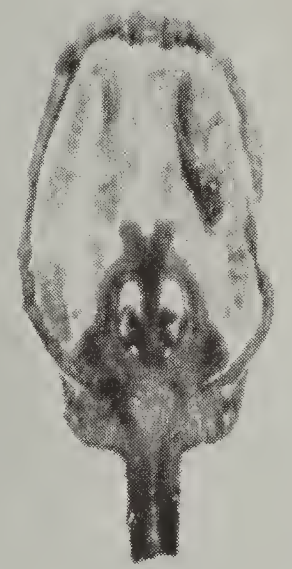

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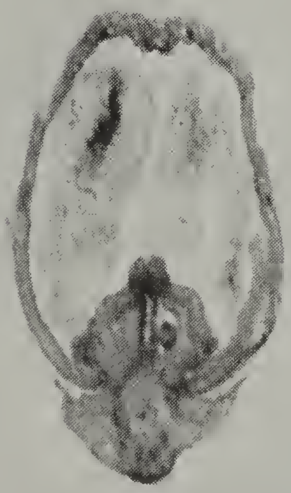

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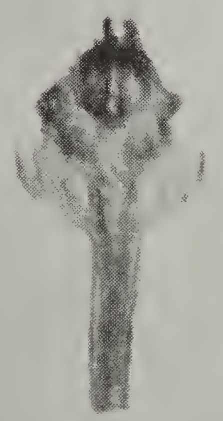

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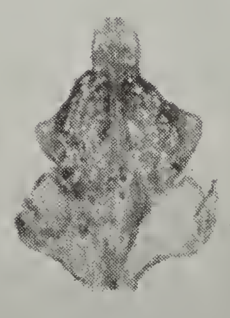

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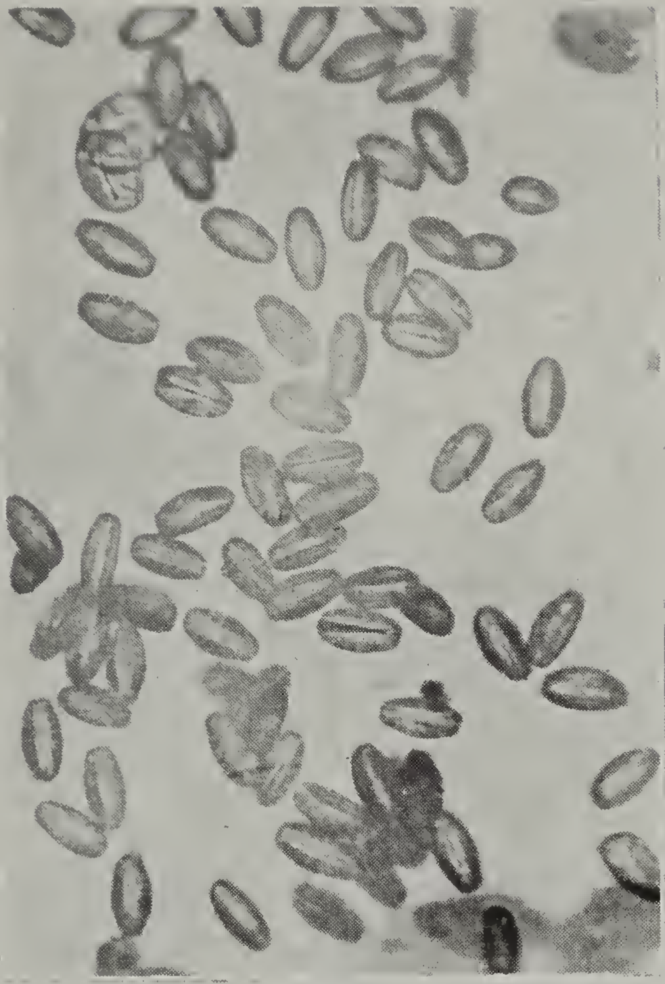
PLATE III

Photomicrographs of pollen grains of Vitis rotundifolia

FIG. 1. Pollen from Hope
FIG. 2. Pollen from Thomas
FIG. 3. Pollen from hermaphrodite seedling with upright stamens, F_1 generations of Thomas × Hope
FIG. 4. Pollen from hermaphrodite seedlings with reflexed stamens, F_1 generation of Thomas × Hope

PLATE III



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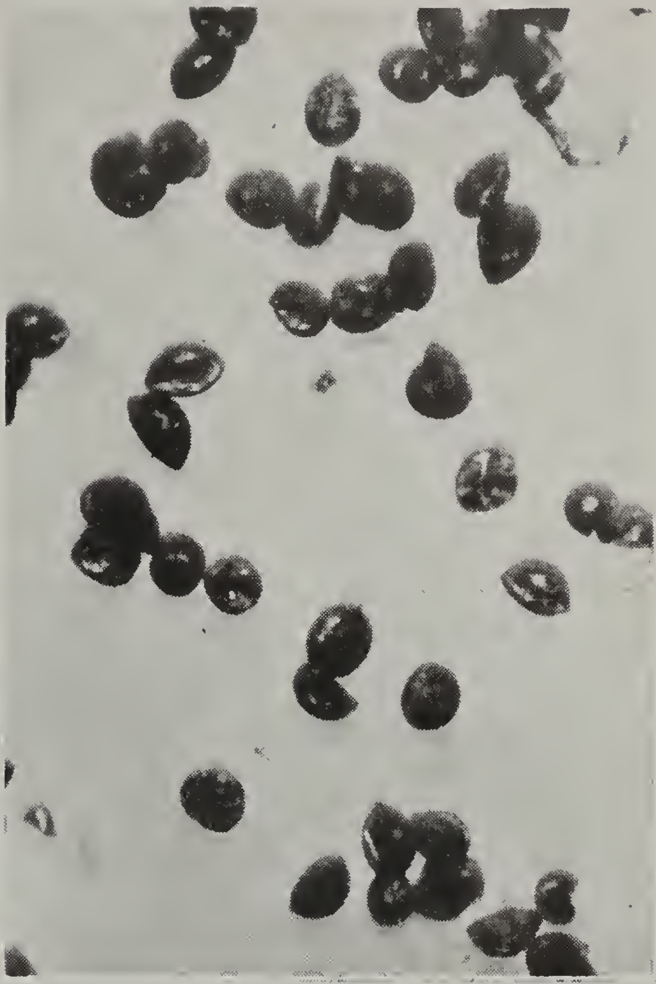

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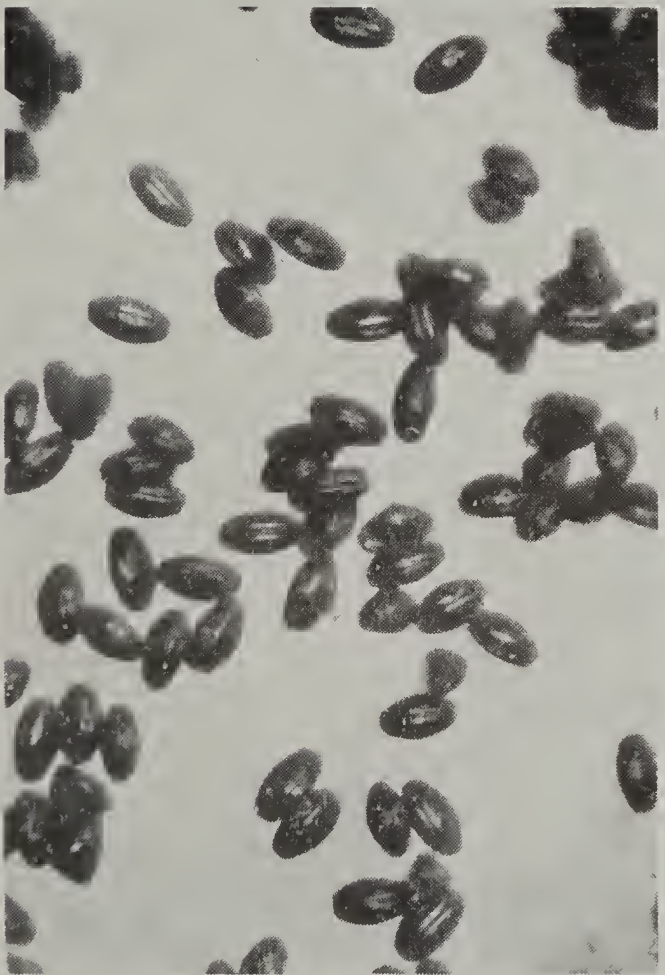

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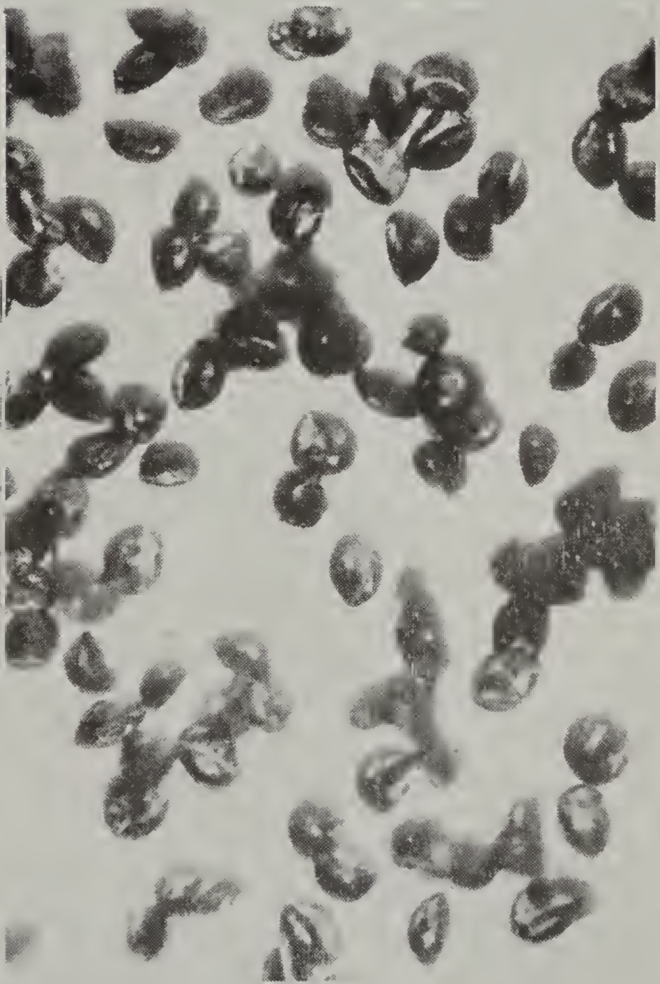

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PLATE IV

Photomicrographs of pollen grains of Vitis rotundifolia

Fig. 1. Pollen from a staminate vine, Dark Male No. 1

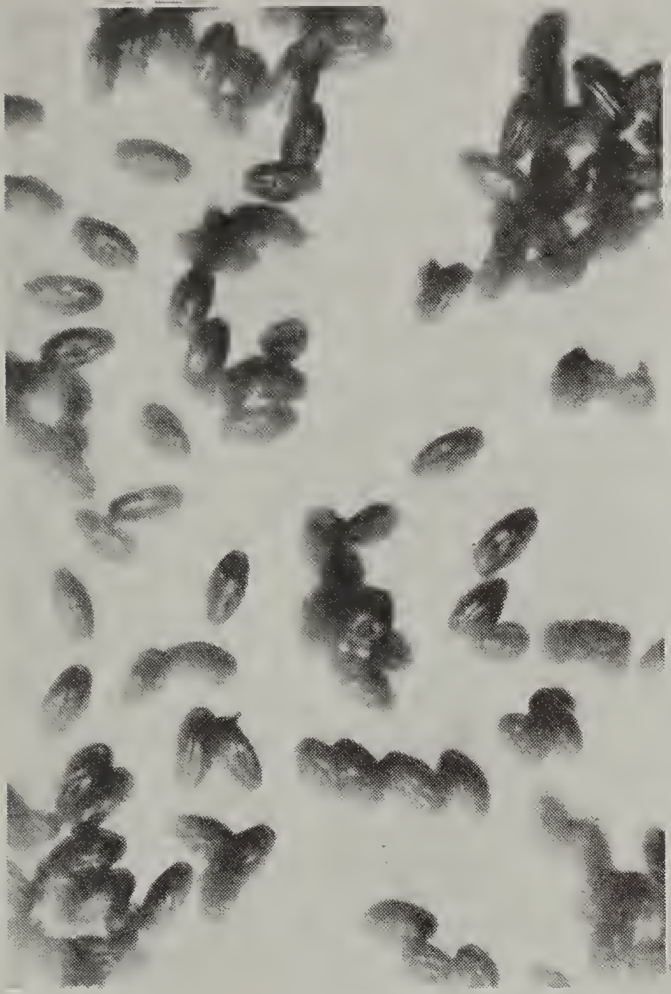
Fig. 2-4. Pollen from the three types of vines of the F_1 generation when Hope is selfed

Fig. 2. Pollen from a staminate vine

Fig. 3. Pollen from a hermaphrodite vine with upright stamens

Fig. 4. Pollen from a hermaphrodite vine with reflexed stamens

PLATE IV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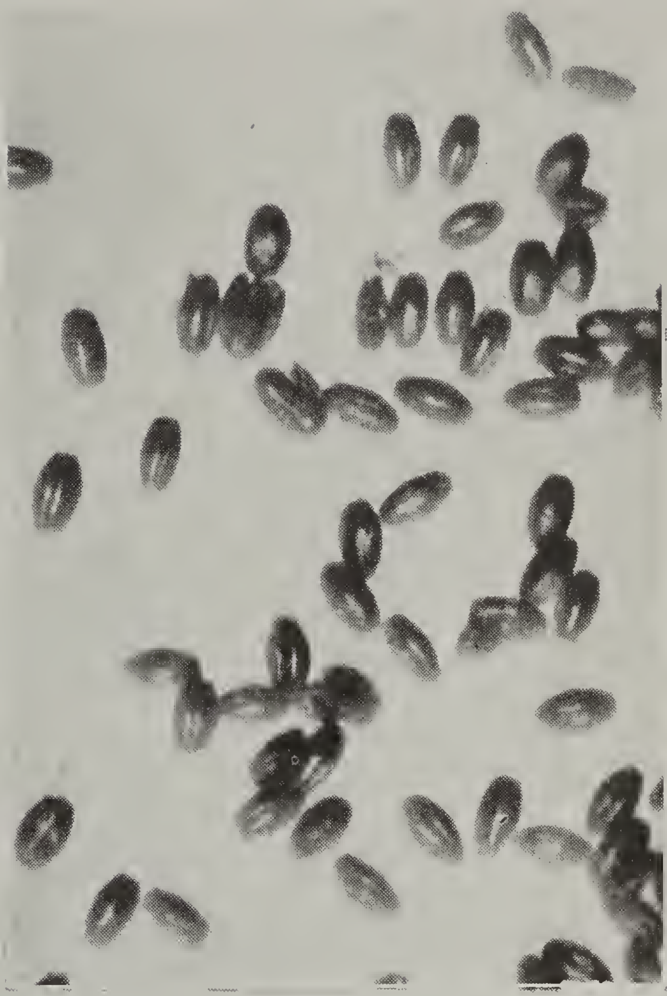
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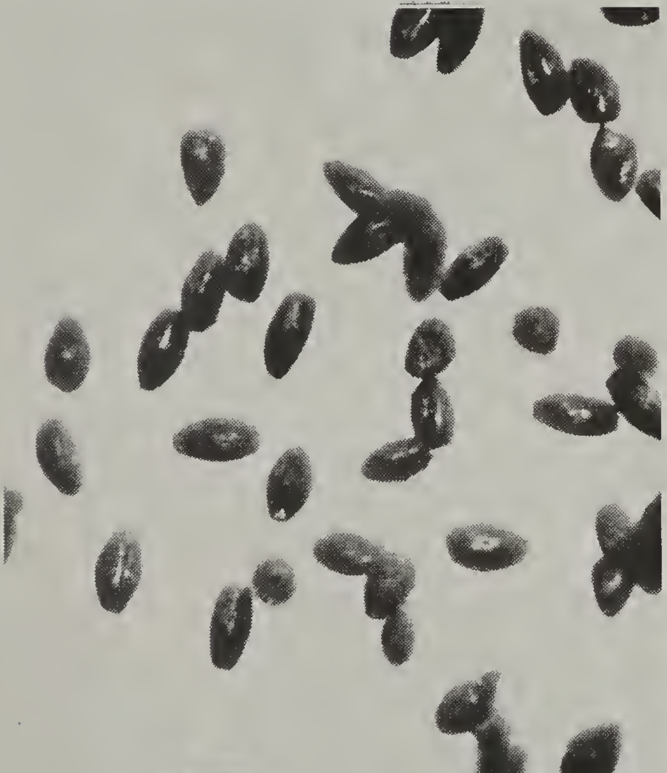
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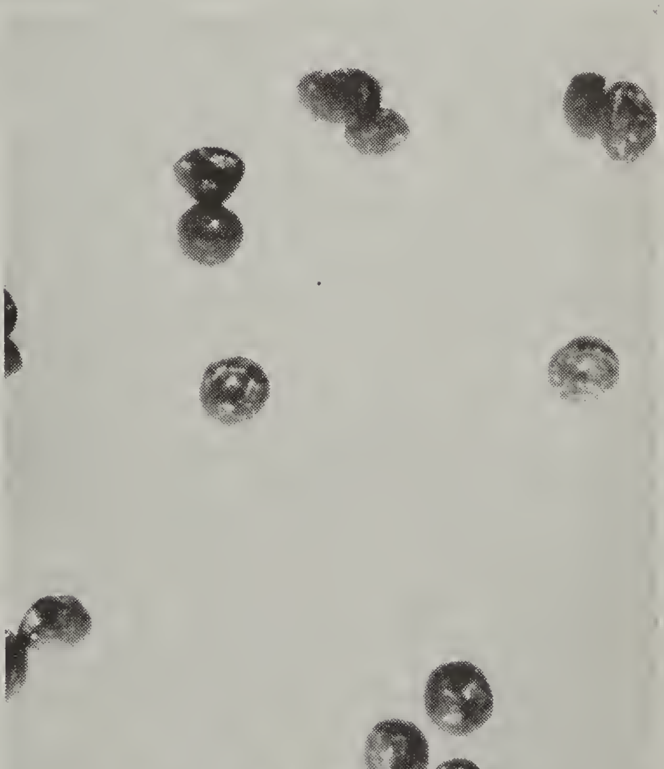
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JANUARY, 1917 TECHNICAL BULLETIN 13

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AND THE
NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING
RALEIGH AND WEST RALEIGH

BIOLOGICAL INVESTIGATION OF SPHENOPHORUS CALLOSUS OLIV.

Z. P. METCALF

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DESCRIPTION OF COLOR PLATE

Figure 1. Stalk of corn showing how the corn bill bug, feeding near the ground, eats holes in the leaves when they are rolled up, so that when they unroll rows of holes show across the leaf. Adult in normal feeding position.

Figure 2. Stalk of corn showing the stunting caused by the larvae working in the tap-root.

Figure 3. Corn bill bug laying egg in cornstalk.

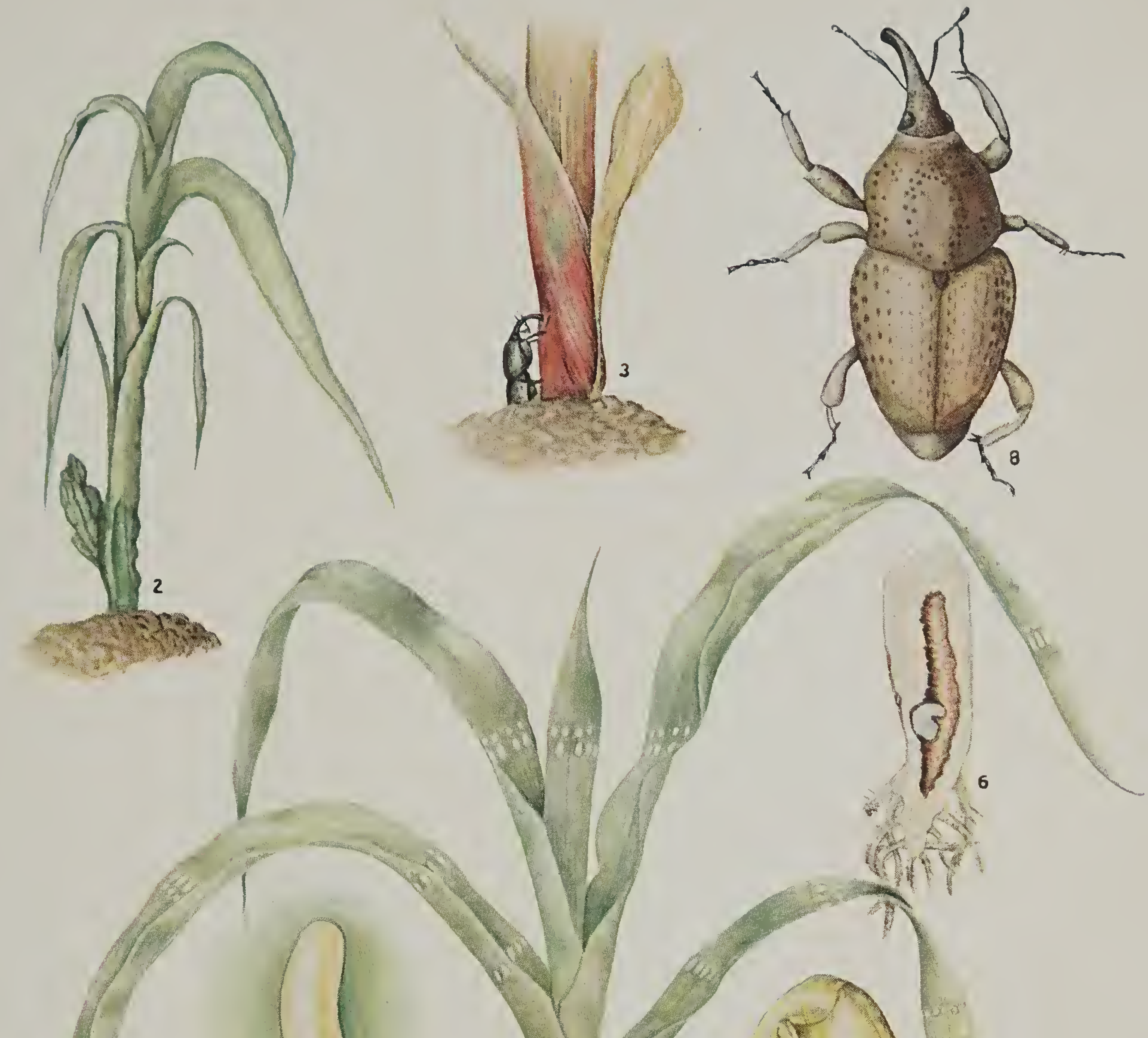
Figure 4. Egg of corn bill bug, much enlarged.

Figure 5. Larva of corn bill bug, enlarged.

Figure 6. Larva in tunnel in tap-root of corn.

Figure 7. Pupa of corn bill bug, enlarged.

Figure 8. Adult corn bill bug, enlarged.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PREFACE

The corn bill bug has long been recognized as one of the important insect enemies of corn in this State. Farmers generally are fairly well acquainted with its ravages, although, needless to say, it is not unusual to find the work of this insect confused with the work of other insects which injure the same parts of the plant.

Recognizing the importance of this pest and appreciating the lack of definite knowledge of this insect, Mr. R. I. Smith, then Entomologist of this Station, began an investigation of the corn bill bug in June, 1910, under an Adams Fund Project, entitled "A Biologic Study of the Species of the Genus Sphenophorus in North Carolina." Mr. Smith continued the investigation of this insect until he severed his connection with the Station in the fall of 1911. A report of Mr. Smith's investigations may be found in the Thirty-fifth Annual Report of the North Carolina Agricultural Experiment Station for the year ending June 30, 1912, pages 105 to 135.

The writer took up active work on the corn bill bug project when he was appointed Entomologist in the spring of 1912, and continued working actively on this project until the fall of 1915. Needless to say, the writer has drawn extensively upon Mr. Smith's paper in writing this bulletin, as Mr. Smith's work and the writer's have formed separate parts of one project. Mr. Smith's work was done largely in the laboratory and insectary. The writer's has been about equally divided between the laboratory and the field. Our results do not always coincide perfectly, due, perhaps, as much to methods and angle of approach as to any factor. Certainly, where conflicting reports are found it will be discovered on inspection that they are well within the probable error for such results.

This investigation has covered in all about five years, and while there are some phases of the question that are still undecided, it is thought that the investigation has reached such a stage that a report upon the biologic and economic aspects of the investigation is entirely justified, leaving a discussion of the other phases of the problem until another time.

TABLE OF CONTENTS

History, synonymy and classification 5-9
Distribution 9-10
Destructiveness 10-12
Common names 13
Insects mistaken for the Southern Corn Bill Bug 13-16
Food plants 16-18
Life history summary 18-22
The egg
    Description 22-23
    Time of hatching 23-29
    Where laid 29-30
    Number laid 30-44
    When laid 44
    Hatching 44-45
    Percentage hatching 45-46
The larva
    Description 46-48
    Methods of rearing 48-51
    Process of molting 51-52
    Number of molts 52-58
    Duration of molts 58-68
    Injury caused by larvae 68-69
    Habits 69-76
    Morality 76-77
The pupa
    Description 77
    Size 77-80
    Pupal cells 80
    Effects of moisture 80-81
    Mortality 82
    Duration 82-88
    Transformation 89
    Habits 89-90
The adult
    Description 90-92
    Duration of life 92-93
    Proportion of sexes 93-94
    Appearance in spring 94-95
    Diurnal activities 95-98
    Habits 98-100
    Oviposition 100-103
    Effects on plant 103-106
Hibernation 106-111
Natural enemies 111-114
Control 114-116
    Time of planting 116
    Rotation of crops 116-118
    Fertilization 118-120
    Drainage 120
    Ridging 120-121
    Fall and winter plowing 121
    Thorough cultivation 121-122
    Destruction of native food plants 122
Bibliography 123

BIOLOGICAL INVESTIGATION OF SPHENOPHORUS CALLOSUS OLIV.

BY Z. P. METCALF

HISTORY, SYNONYMY, AND CLASSIFICATION

The corn bill bug (*Sphenophorus callosus Olivier*) belongs to the family *Curculionidæ*, the sub-family *Calandrinæ of the sub-order Rhynchophora.* This family includes various species of bill bugs proper, many of which are of economic importance in the United States, preying upon corn, grasses, etc., and the rice and granary weevils which feed upon various kinds of stored products and grains.

Blatchley and Leng (1916) describe the sub-family *Calandrinæ* as follows: "A rather small group of usually large, robust species, having the antennæ elbowed, inserted near the base of beak, their grooves very short, not receiving the scape; funicle 6-jointed, club not annulated, shining; labrum wanting; mouth cavity elongate, peduncle of mentum narrow, elongate, concealing the oral organs; mandibles compressed, with three apical teeth; beak variable in length and sculpture; thorax truncate in front and beneath, without ocular lobes; elytra without epipleurae and with a strong fold on inner face; abdomen with five ventral segments, the first and second longer, the third and fourth short, their sutures straight and deeply impressed, fifth equal to third and fourth fitting into the groove of the underside of elytra; last spiracle covered by the ventral segments; pygidium large, nearly perpendicular, exposed in both sexes; last dorsal of male quadrate and more or less retracted or concealed; coxæ all more or less separated, the hind ones transverse, oval; femora usually strongly clavate, not toothed; tibiæ short, not serrate, clawed at the outer angle; tarsi rarely brush-like beneath, third joint rarely bilobed; claws divergent, simple.

"The larvæ of the larger species bore into the stems of plants, especially grass and corn, while those of the smaller ones infest seeds and grain."

The genus *Sphenophorus* Schoenherr is world-wide in distribution, containing species that are of economic importance in several countries. It was described in 1837 by Schoenherr as follows:

Genus 390. Sphenophorus nobis.

Rhynchophorus Herbst. Schh. (1) Say—Calendra Clairville, Illiger, Germar; (2) Calandra Fabr. Olivier, Latreille, Germar, Dejean, Illiger, Oken, Sturm; (3) Curculio Linne et plures.

Character generis—Antennæ mediocres validiusculæ articulis duobus basalibus funiculi oblongis turbinato-obconicis, reliquis brevibus, sub- rotundatis omnibus distanibus clava breviter ovata, compressa, cuneiformis.

Rostrum elongatum, sub tenue, basi crassius, modice arcuatum, Thorax oblongus, antic angustior valde coarctatus, basi aut bi-sinuatus, aut rotundatus.

Elytra oblonga, sub ovata, apice in pleurisque singulatim rotundata, supra saepissime planiuscula.

Pedes mediocres, validi, longitudine sub aequales.

DESCRIPTION—Corpus elliptico-ovolum, supra aut planiusculum, aut paraum convexum, alatum, plerumque mediae magnitudinis. Antennae thoracis medium pertingentes, prope basim rostri insertae, validusculae, fractae, articulatae, scapo elongato thoracem apice attingente, sub-clavato; funiculo 6-articulato; articulis, 1, 2 oblongis, turbinato, obemicis, 3-6 in pleresque sub-rotundatis in caeteris sub-turbinatis, exterioribus successive parum crassioribus, omnibus distantibus; clava breviter ovata, compressa, cuneiformis bi-articulata; articulo 1° corneo, apice truncato, 2° spongroso, apice acetoto, aut truncato, aut sub-rotundato. Rostrum fere longitudine throacis, ante insertionem antennarum crassius dein sub-tenne, arcuatum, deflexum. Ocli lalerales, infra caput fere connexi, oblongi depressi. Thorax lalitudine baseos longior in plerisque bisinuatus, in allis rotundato productus, lateribus in plerumque basi sub-rectis, dein versus apicem porun rotundatis; anterius angustior, in nonnullis magnis aequaliter rotundatus, anticc subito angustior, semper longe intra apicem profunde, coaratus collun quasi annuliforme formans convexus. Scutellum triangulare. Elytra antice thoracis basi parum latiora et illa fere sesqui-longiora basi partim singulatim, sub-rotundata, partim conjunctium introsum rotundata—emarginata pone basim oblique ampliata dein posterius attenuata apice obtutus rotundata, aut sub-trunctata, abdomine breviora, supra in plerisque planiuscula in paucis nonnihil convexa. Pygidium sub-triangulare, deflexum. Pedes mediocres, longitudine sub-aequales validi; femoribus clavatis, saepissiare muticus raro sub-tusfixo, valido terminatis tarsi, elongatis, articula penultima, aut majusculo, subtus spongioso, out paulo minori subtus haud spongioso, unguiculari elongato clavato bi-inguiculata.

Typhus Sphenophorus abbreviatus.

(Patria Totus orbis; præsertim vero regiones calidæ.)

OBSERY—In numeroso hoc genere occurunt quedem species nonnulae, omnibus supra indicatis characteribus non exacte convenientes; dum vero notis generis maxime essentialibus correspondent, illas separare minus necesse censui. Inveniuntus elim-aliae quarum forma corporis paulo convexior et tarsi diversimoda constructi pro quibus, ut interim saltem stirpem peculiaren assignavi.

Riley (1881) gives the following as the distinguishing characters of the genus:

"The distinguishing generic characters of *Sphenophorus* may be given briefly as follows: Side pieces of metasternum rather narrow; epimera of mesosternum externally truncate (not acute); front coxæ narrowly separated by the prosternum; third joint of the hind tarsi either glabrous or only pubescent on the sides. A peculiar external appearance will render the genus at once recognizable to the experienced eye, while the numerous species are very difficult to distinguish. The form of the tibia and tarsi and the vestiture of the latter have furnished excellent characters to divide the genus into natural groups. That to which our species (robustus) belongs is characterized as follows:"

Blatchley and Leng (1916) decribe the genus *Sphenophorus* as follows: "Rather large, robust, usually elliptic-ovate species, having the body glabrous, often covered with a clayey artificial coat so as to hide the sculpture, more rarely with a dense natural glabrous coating; antennæ inserted near base of beak, scape long, slender, funicle six-jointed; club wedge-shaped, convex in front, the outer third of more sensitive; beak shorter than thorax, rather slender, feebly curved, swollen at base; antennal grooves very short, fovea-like, located close to eyes; thorax longer than wide, its disc usually with elevated smooth lines or spaces; elytra usually wider than thorax, their tips separately broadly rounded, thus widely exposing the pygidium."

The species that we are interested in was described by Olivier in 1807 as *Calandra callosa* from "Carolina," a region of great extent and including the greater part of the range of this species as we know it today. Olivier's description is as follows:

> 27 Calandra calleuse, Calandra callosa, Charauson, pl. 28 Fig. 416. C. d un noirgris obscur; elytres avec un point calleux lursant. C. obscura cincerascens, elytris puncto culloso nitido.
>
> Antennæ nigræ apice cinero. Rostrum nigrum basi fusco-cinereum. Thorax fusco-cinereus obscurus dorso cruce vix elevata. Elytra haud striata subvariolosa fusco-cinerae obscura callo postico nitido. Corpus subtus pedesque fusco-cinerae obscura.
>
> Tout le corps de cet insecte est d 'un noircendre obscur. Les antennes sont d 'un noir frun, luisant, avec le base condrec, obscura. Le corcelet est inegal et un voit a sa portie superceure une elevation en croix pen marquec les elytres sont inegales, presque variolees, marquecs, vers l'extremite d 'un point calleux presque epineux, noiratie, lursant.
>
> Elle se trouve dons la caroline.
>
> Du cabinet de M. Bosc.

In 1837 Schoenherr redescribes this species in his "Genera et Species curulionidum" as follows:

> 71 S. CALLOSUS OLIVIER.
>
> Ellipticus, niger, opacus, indrimento fusco-cinereo, undique incustatus; thorace, inaequali, varioloso-punctato plagis tribus elevatioribus, natato media sub-cruciformi, elytris obsolete, remote punctato striatis, callo postico elevata nitido.
>
> Calandra Callosa Oliv. Ent. V. 83, pp. 92, 27.
>
> Patria America Septenbronalis.

This species seems not to have been referred to again until 1873, when Le Conte and Horn in their Rhynchophora of America North of Mexico unite this species with *S. cariosus* Oliv., stating their reasons for so doing as follows:

"Dr. Horn has suggested to me that this species and *S. callosus* Oliv. should be united. After careful examination of the specimens in my collection, I think this view is correct. Those who are inclined to adopt it will place *Callosus* as the synonym, since it is represented by old and abraded specimens."

S. callosus Oliv. was therefore considered a synonym of *S. cariosus* Oliv. until 1906, when Chittenden (1906) cleared up the situation by pointing out that callosus must be considered as distinct from cariosus. That portion of his paper which refers to callosus is as follows:

SPHENOPHORUS CALLOSUS OLIVIER.

Calandra callosa Oliver, Hist. Nat. des Ins., Vol. V., p. 92, Pl, XXVIII. Fig. 416, (1807).

Sphenophorus sculptilis Horn (nec Uhler). Proc. Am. Phil, Soc., p. 424 (1873).

This species was united by Le Conte and Horn with *cariosus*, but wrongly so, as I shall attempt to prove. Olivier's description reads in substance as follows:

"Body black with dark cinereous coating. Antennæ brownish black, shining, cinereous at apex. Rostrum black, *dark cinereous at base.* Thorax uneven, and one sees on the superior portion an elevation in the form of a cross, feebly marked." Elytra uneven, feebly variolate, marked toward the apex with a callous point, nearly spinose, blackish, shining.

"Olivier's illustration is imperfect in that it is very crude, showing neither punctuation nor sculpture, and the general impression is that of a shining species, which was certainly not intended. The thorax is a little short, otherwise the form coincides with the species which is figured herewith.

"The cinereous base of the rostrum is an important character, as it signifies that a considerable portion of the base is coated, while in *cariosus* it is not. The cross-like elevation of the thoracic disk is aptly described as *feebly* indicated; in fact, it requires little imagination to discern it in many individuals; moreover, it is not shown in Olivier's figure. In the true *cariosus* the cross is black, shining, and well marked, and the cariniform base of the third elytral interval which is always present is so prominent as to attract the attention of the veriest tyro.

"The type locality 'Carolina' will answer for either species. North Carolina appears to be the metropolis of *callosus*."

Blatchley and Leng (1916) described *Sphenophorus callosus* Oliv., as follows:

"Oval robust. Black, densely clothed with a brownish or olivaceous clayey coating; antennæ and tarsi reddish-brown. Beak two-thirds the length of thorax, compressed and sparsely punctate except near base, where it is swollen, coarsely punctate, and shallowly grooved above. Thorax slightly longer than wide, sides parallel from base for three-fourths their length, then strongly rounded to the constricted apex; disc very coarsely and irregularly punctate, the median vitta usually broadly dilated at middle, its apical portion very narrow; lateral vittæ broad at base and with a short oblique branch, their front portion often replaced by coarse punctures. Elytral oval, their surface uneven, sides gradually narrowed from humeri to apex; striæ fine, coarsely and distinctly punctate; intervals flat, their punctures hidden, the third sometimes feebly base. Humeral umbone and sub-apical callus somewhat prominent, often shining. Under surface coarsely and sparsely punctate. Length, 8-12 mm."

The synonym of this species would therefore seem to be as follows:

1807. *Calandra callosa* Olivier. Hist. Nat. des Ins. Vol. 5, p. 92, pl. 27, fig. 416.

1837. *Sphenophorus callosus* Olivier. Schoenherr Genera et species curculionidum, page 492.

1873. *Sphenophorus sculptilis* Horn (nec Uhler). Rhynchophora of America North of Mexico, page 424.

1873. *Sphenophorus cariosus* Le Conte and Horn. Rhynchophora of America North of Mexico, page 425.

1906. *Sphenophorus callosus* Chittenden. Proc. Ent. Soc., Wash. Vol. 7, pp. 166-182.

DISTRIBUTION

The distribution of the corn bill bug as recorded by Blatchley and Leng (1916) is as follows: Maine to Florida, westward to Arizona and northern Mexico and nothward to Wisconsin (Fig. 1).

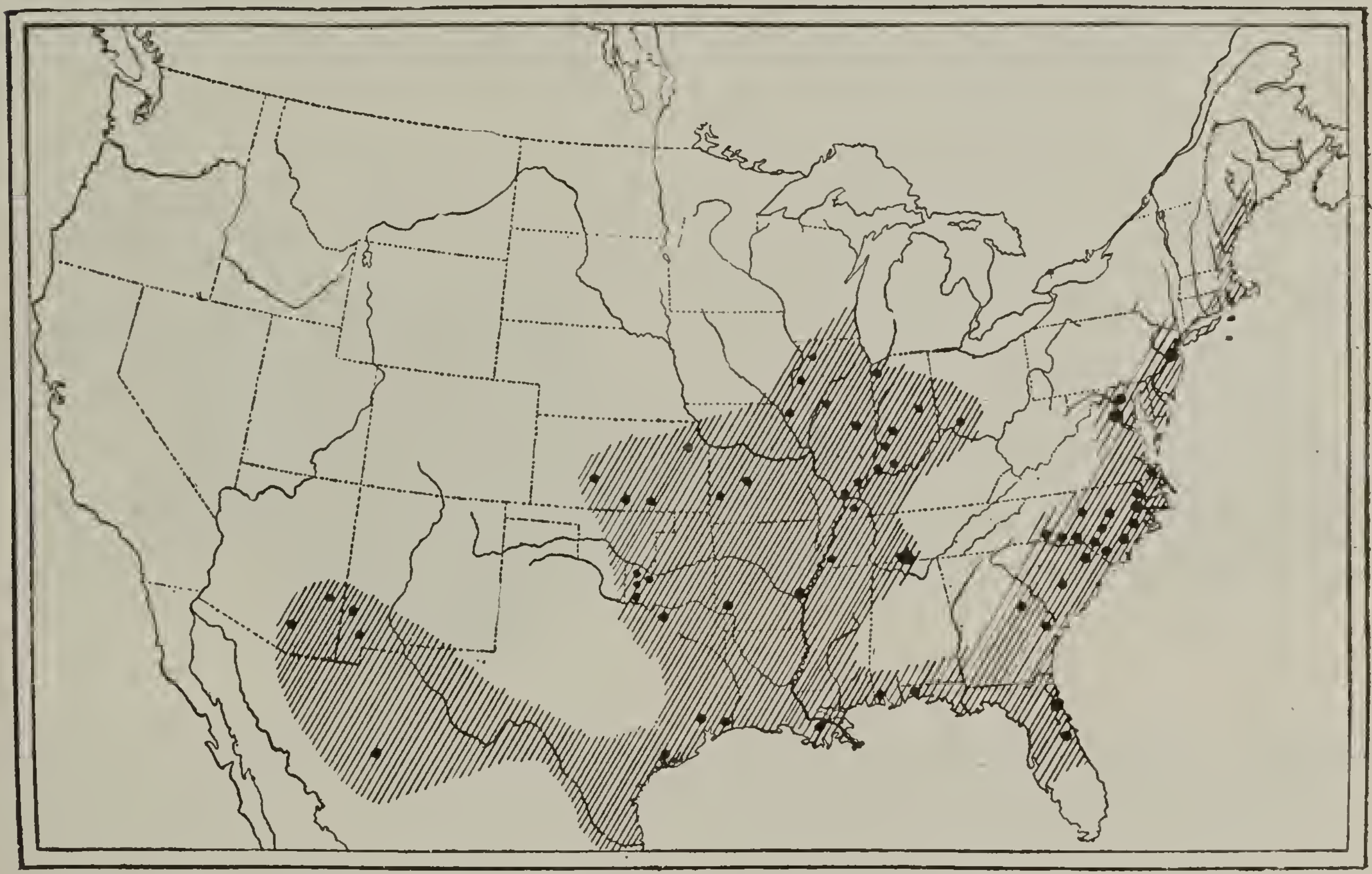


FIGURE 1. Map of the United States showing the distribution of the Southern Corn Bill Bug. Dots show recorded distribution. Shaded area approximate distribution. (In part after Webster.)

In this State it is largely confined to the region known as the Coastal Plain. This in a general way corresponds to the Lower Austrial Life Zone. Its distribution in the State as determined by the records of the United States Bureau of Entomology and the records in this office are shown graphically in Fig. 2. The dots show places where this insect has been found by field investigations or by correspondence. The shading indicates the approximate distribution of this species in the State.

In the map (Fig. 2) the area of especial destructiveness to corn is shown by double shading.

In the writer's experience, this insect is almost universally present in the eastern part of its range. In the western part of its range, however, it is confined to the low swampy situations along streams. This would indicate very strongly that the insect is very dependent upon soil with a great amount of water for its best development.

In the western part of its range it may be as abundant on cyperus as in similar situations in the eastern part of its range, and although corn is frequently planted in close proximity to such low swamps, the corn bill bug does not, so far as our observations go, turn its attentions from the cyperus to the corn.

DESTRUCTIVENESS

Probably no corn insect causes as great loss both directly and indirectly in the eastern part of this State as the corn bill bug. Complete destruction of entire fields is not only frequent, but common. Farmers frequently replant such fields in the hope of getting a stand, but it is not at all unusual for the third and often for the fourth planting to be so badly injured as to cause the farmer to abandon the field entirely.

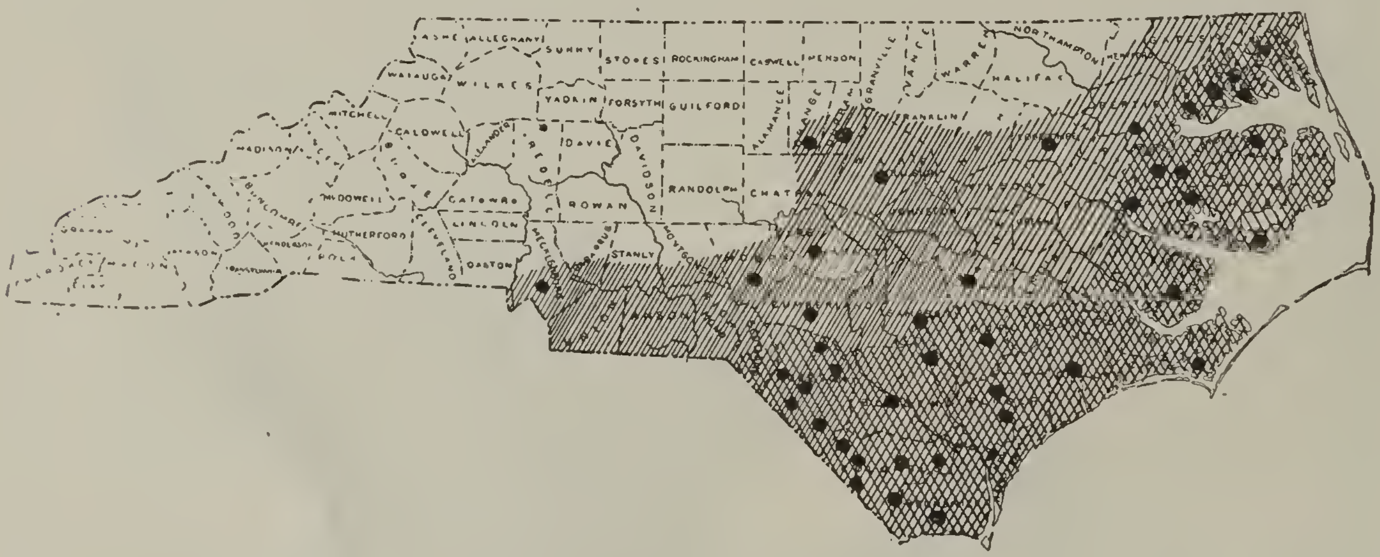

ASHE
ALLEGHANY
SURRY
STOKES
ROCKINGHAM
CASWELL
PERSON
GRANVILLE
VANCE
WARREN
NORTHAMPTON
HALIFAX
WATAUGA
WILKES
YADKIN
FORSYTH
GUILFORD
ALAMANCE
ORANGE
FRANKLIN
MITCHELL
CALDWELL
MADISON
DAVIE
DAVIDSON
RANDOLPH
CHATHAM
McDOWELL
CATAWBA
ROWAN
LINCOLN
RUTHERFORD
CLEVELAND
GASTON
MECKLENBURG
CABARRUS
STANLY
MONTGOMERY
ANSON
POLK
HENDERSON
BUNCOMBE
MACON

FIGURE 2. Map of North Carolina showing the distribution of the Southern Corn Bill Bug. Dots show actual records. Shaded portion approximate distribution. Doubly shaded portion area of greatest destruction.

As swamps are drained and put under cultivation the usual practice is to farm to corn for the first few years. The corn bill bug, driven from its natural food plant, turns its attention to the corn, and does so much damage that the growing of corn under such conditions is rendered entirely without profit.

A general conception of the appearance of such fields may be gathered from an examination of Figs. 3 and 4. In really serious cases of damage from this insect practically every stalk will be destroyed or only a stalk here and there will escape destruction. In cases of milder amount of damage more stalks will escape destruction, but a great many stalks will be so badly deformed that they make no satisfactory growth and never produce ears. A few stalks more favorably located will make a fair growth and will stand above their neighbors like giants among dwarfs.

The very irregular growth of the stalks in the field is perhaps the best indication of the presence of the corn bill bug. Other indications are the rows of very regular holes across the leaves, and the presence of the adults clinging to the base of the stalk and the presence of the larvæ in the stalks and among the roots.

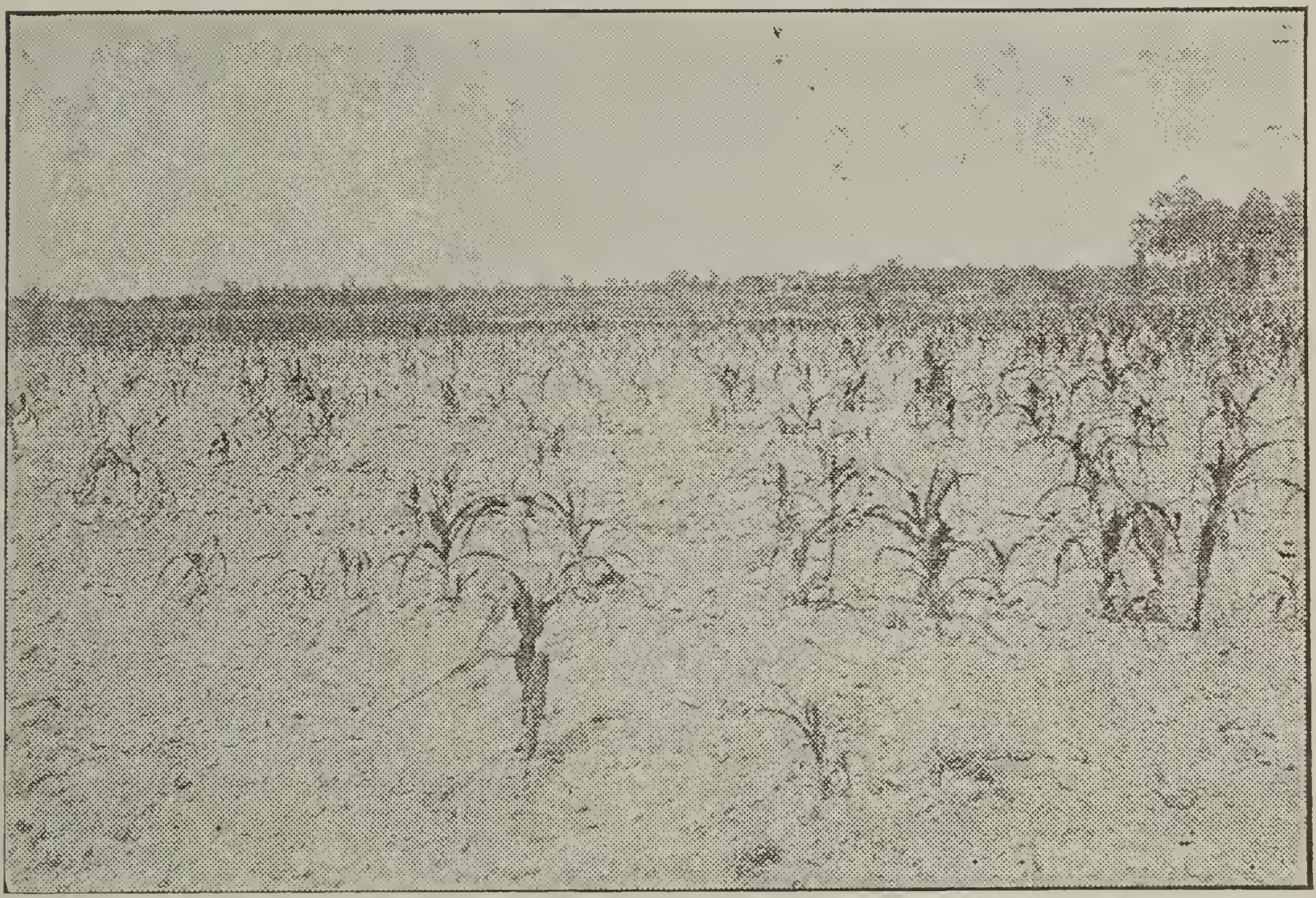


Figure 3. Field of corn, showing characteristic injury by Southern Corn Bill Bug.

In fields examined in this State the writer has tried as far as possible to determine the relative amount of damage that has been caused by corn bill bugs under different conditions, and while no entirely satisfactory method has ever been devised for expressing the amount of damage caused under different conditions in different fields, he has as far as possible tried to express this in a rate per cent, basing his conclusions upon a careful stalk-to-stalk examination of representative areas and also by a summary of the field as a whole after a careful survey.

Needless to say, a stalk-to-stalk inspection alone will not be an entirely satisfactory basis for estimating damage, for very frequently stalks are damaged so slightly by the adult beetles that the stalk will afterwards almost completely recover. This is especially true where the total number of beetles is small and the percentage of stalks damaged is relatively small.

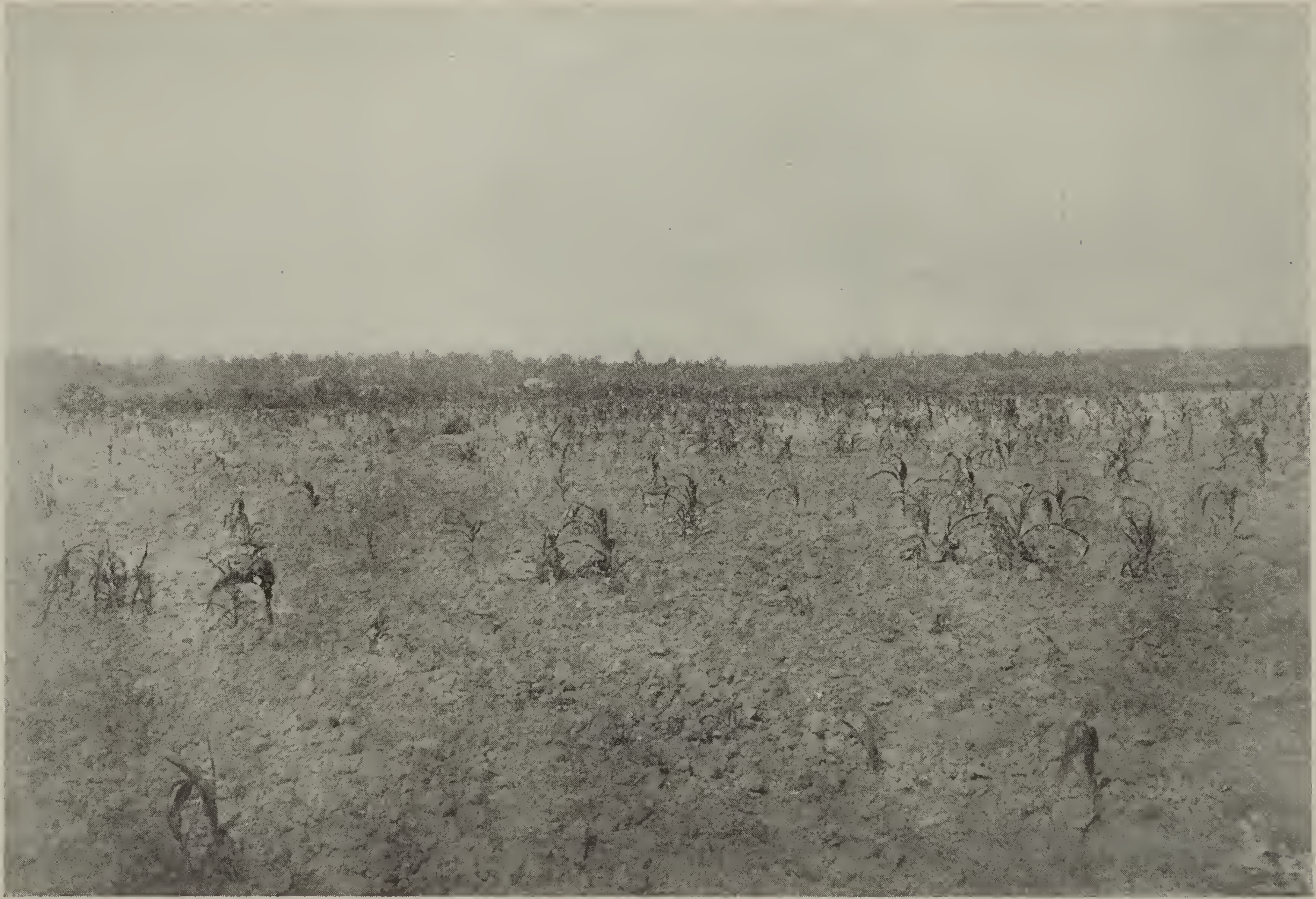


FIGURE 4. Field of corn, showing injury by Southern Corn Bill Bug.

COMMON NAMES

The southern corn bill bug has a large number of names in this State, the most common being derivatives of the name "Curlew Bug." The name "Curlew Bug" is given on account of its fancied resemblance to the Curlew. The curlew was a fairly common bird on our coast with a long curved bill resembling to a certain extent the snout of the corn bill bug. Some of the more common corruptions of the name "Curlew Bug" are "Klew" or "Clew Bug" and "Curlie Bug." It is also generally known as "bill bug" and "corn bill bug." The rice growers speak of it familiarly as "Rice Bug" and "Rice Bill Bug." In other sections it is known almost exclusively as "Chufa Bug" from its habit of feeding to a great extent upon the chufa. Other names may be used in various localities, but in the writer's experience these names are the ones most commonly used.

In this report the writer has adopted the name Southern Corn Bill Bug to distinguish it from other corn bill bugs found in other parts of the country. The writer feels that the selection of this name needs no special defense. He has selected it in place of the more common "Curlew Bug" for the reason that it calls to one's attention at once that this is the bill bug that is especially destructive to corn in the Southern States which the name curlew bug does not suggest.

INSECTS WHICH ARE OFTEN MISTAKEN FOR THE SOUTHERN CORN BILL BUG

There are in this State many different kinds of snout beetles, any one of which is apt to be mistaken by the farmer for the Southern Corn Bill Bug; but so far as the writer is aware, none of these snout beetles save the corn bill bug has the habit of feeding at the base of cornstalks, head downward. (Fig. 5.)

The work of various kinds of insects is often mistaken for the work of the corn bill bug. One of the most characteristic things about a plant injured by the adult Southern Corn Bill Bug is the presence of transverse rows of holes across the leaves. At least three other insects in this State also eat rows of holes across the leaves. These are the larger cornstalk-borer, the corn ear-worm, and the adult of the Southern corn root-worm. The work of the Southern Corn Bill Bug may be distinguished from the work of these three insects by its greater regularity. (Fig. 7.) This is due, no doubt, to the fact that the corn bill bug works well down at the base of the stalk in the "bud," whereas the other insects work in the top in the unfolding leaves. Of these three, the work of the larger corn stalk-borers on the unfolding leaves resembles most closely the work of the corn bill bugs. The work of the stalk-borer is never quite so regular, however, as the corn bill bug's work, and frequently the rows of holes are only on one side of the heavy mid-rib, whereas in the case of the corn bill bug they are on both sides of the leaves and usually they are of almost the same size and shape on both sides of the leaf. The holes eaten by the corn bill bug are nearly always with smooth round edges, as if they had been cut

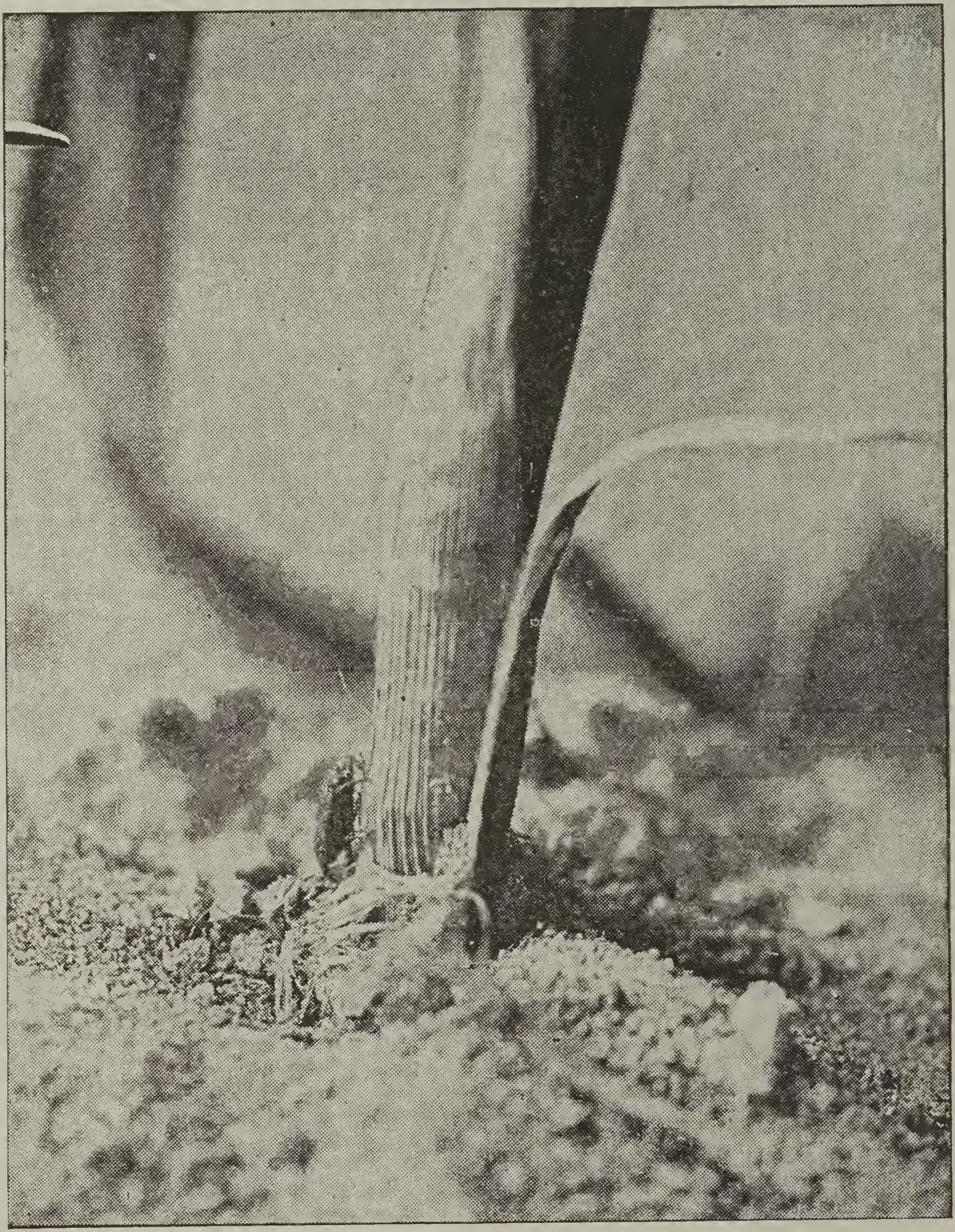


FIGURE 5. Adult Southern Corn Bill Bug feeding on corn, normal position.

with a sharp punch, while the holes eaten out by the corn stalk-borer are usually decidedly more ragged. The holes eaten by the corn earworm are usually not round, but elongate and decidedly more irregular, on the average, than the holes made by the corn stalk-borer. The holes eaten by the adults of the southern corn root-worm have a tendency to be square instead of being round. Not infrequently these beetles eat the tender leaves just above the seed leaf until the unfolding leaves are completely cut off. In this respect their work resembles the work of certain cut-worms, species not determined, which have the habit of crawling up the stalk and cutting off the unfolding leaves. In all the fields examined, however, the work of the adult root-worms was much more common than the work of the cut-worms.

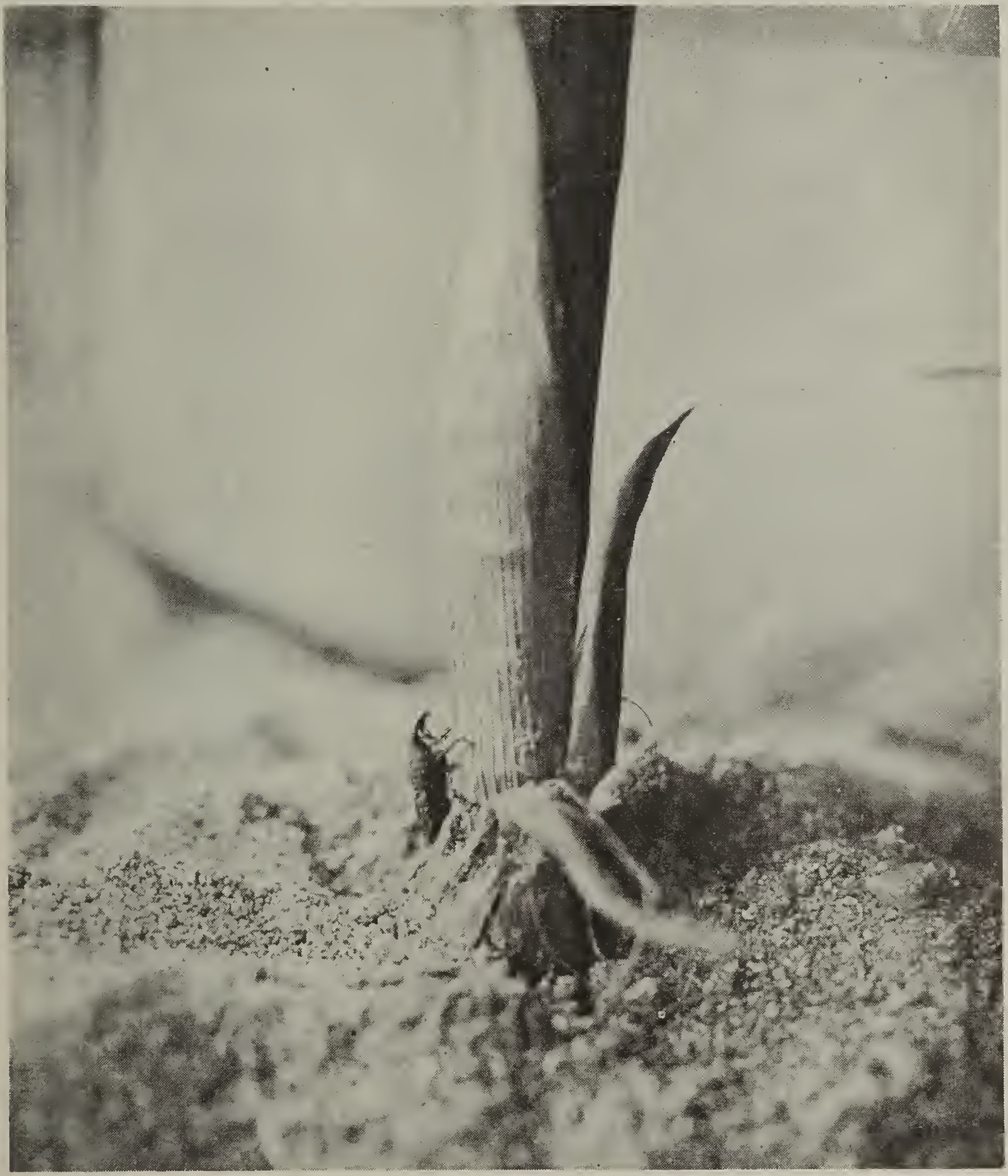


FIGURE 6. Adult female Southern Corn Bill Bug ovipositing in corn, normal position.

In general, farmers do not distinguish clearly between the work of the adult corn bill bugs on young corn and the work of the southern corn root-worm. The work of the corn root-worm is most conspicuous about the time the corn is sprouting. At this time the seed leaf is vigorous and green and the unfolding leaves (bud) are withered and dead in those stalks that have been attacked by the root-worm. (Fig. 9.) On the other hand, in stalks attacked by the corn bill bugs all the leaves remain fresh and green at this time and later the whole plant dies if it is severely attacked. These young stalks usually show the characteristic rows of holes acoss the leaves. (Fig. 10.)

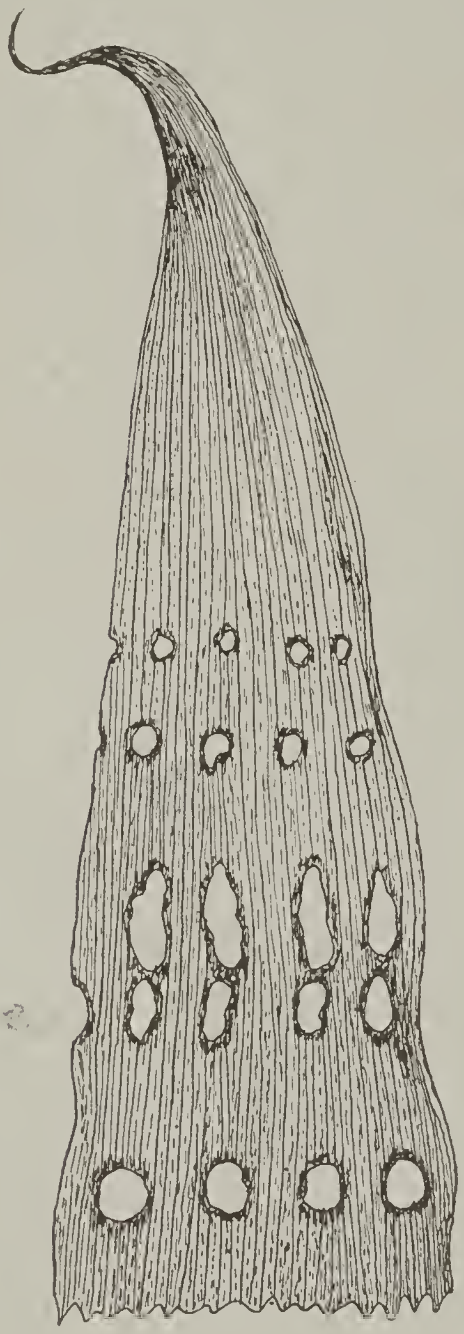


FIGURE 7. Portion of a corn leaf showing characteristic arrangement of feeding punctures.

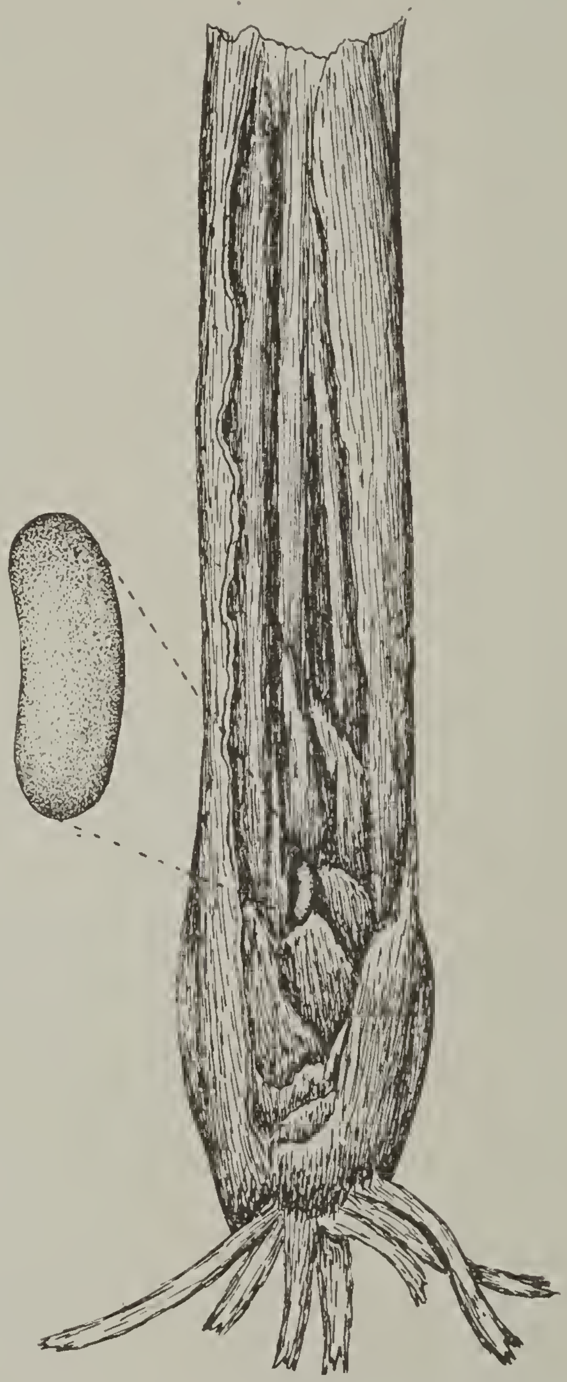


FIGURE 8. Young corn stalk showing normal position of egg in heart, and egg more enlarged.

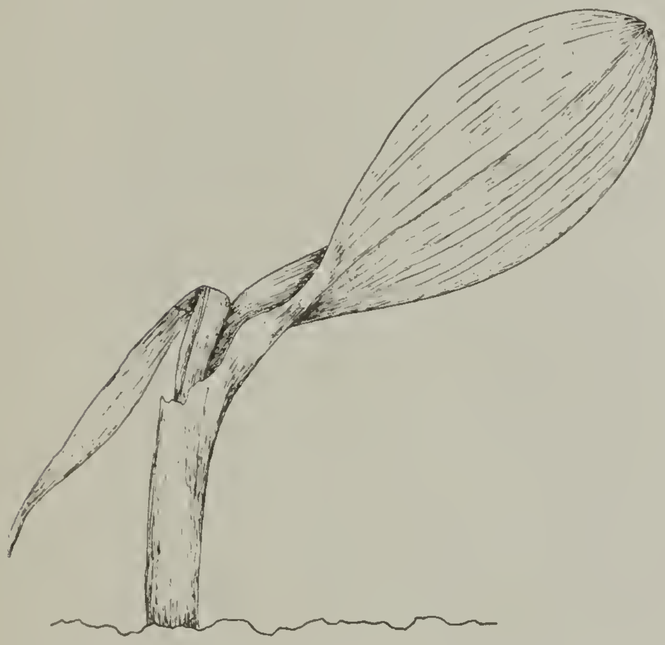
FOOD PLANTS

The following plants have been found infested with the corn bill bug. Corn, rice, and various species of cyperus as follows:

Cyperus flaricomus, at Raleigh and Willard,
Cyperus cylindricus, at Proctorville and Braswell,
Cyperus strigosus, at Lumberton and Chadbourn,
Cyperus overlaris, at Pembroke,
Cyperus esculentus (Chufa).

Of these plants, corn, rice, and chufas are infested wherever they are grown in the bill-bug section of the State, so far as we have observed. Of these plants, rice seems to be the favorite food where it can be secured, followed by chufas and corn. The reason for placing chufas ahead of corn is that around Raleigh, so far as observed, bill bugs are plentiful on chufas, but do not trouble corn even when they are growing side by side. The various species of cyperus will undoubtedly have to be considered as the native food plant of this species in spite of the fact that it has been reared from other grasses (Figs. 11, 12, 13).

According to Webster (1912), the following plants have been reported as food plants for this species from various parts of the country:



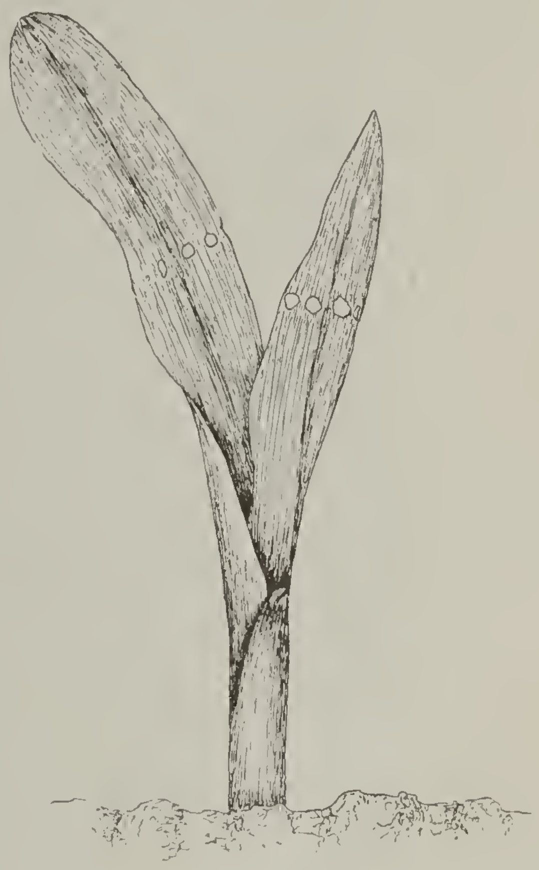


Figure 9. Young corn plant showing injury by Southern Corn Root Worm.

Figure 10. Young corn plant showing injury by Southern Corn Bill Bug.

"Dr. Forbes gives *Cyperus strigosus* as the natural food plant, in the roots of which it develops in Illinois. Mr. T. D. Urbahns found it developing in *Tripsacum dactyloides* at Plano, Tex., in July, 1909. At Appleton, Tenn., July 14, 1911, Mr. George G. Ainslie found the infested fields in part grown up with weeds and a swamp carex (*C. vulpinoidæ*), but he was unable to find the beetle actually developing therein. (See Pl. IX, figs. 1, 2.) Mr. A. N. Caudell reported the larvæ injuring the roots of yellow nut-grass (*Cyperus esculentus*) at Stillwater, Okla., in 1895. Dr. Chittenden reared the adult from a pupa found in the roots of *Panicum capillare* growing in low bottomlands along the canal near Glen Echo, Md., in August, 1897. Mr. I. J. Condit found it breeding in Frank's sedge (*Carex frankii*) growing on the Department farm at Arlington, Va. In Florida the insect develops from egg to adult in *Cyperus rotundatus,* while farther north, in the

Carolinas, the common food plant is the "chufa" (*Cyperus esculentus*). To such a degree is this true in the latter locality that the insect is supposed by farmers to have been introduced with that plant. Quite in accord with the foregoing, Mr. J. G. Sanders reared adults March 30, and again April 25, 1908, from *Cyperus exaltatus,* introduced from Egypt and growing on the Department farm at Arlington, Va."

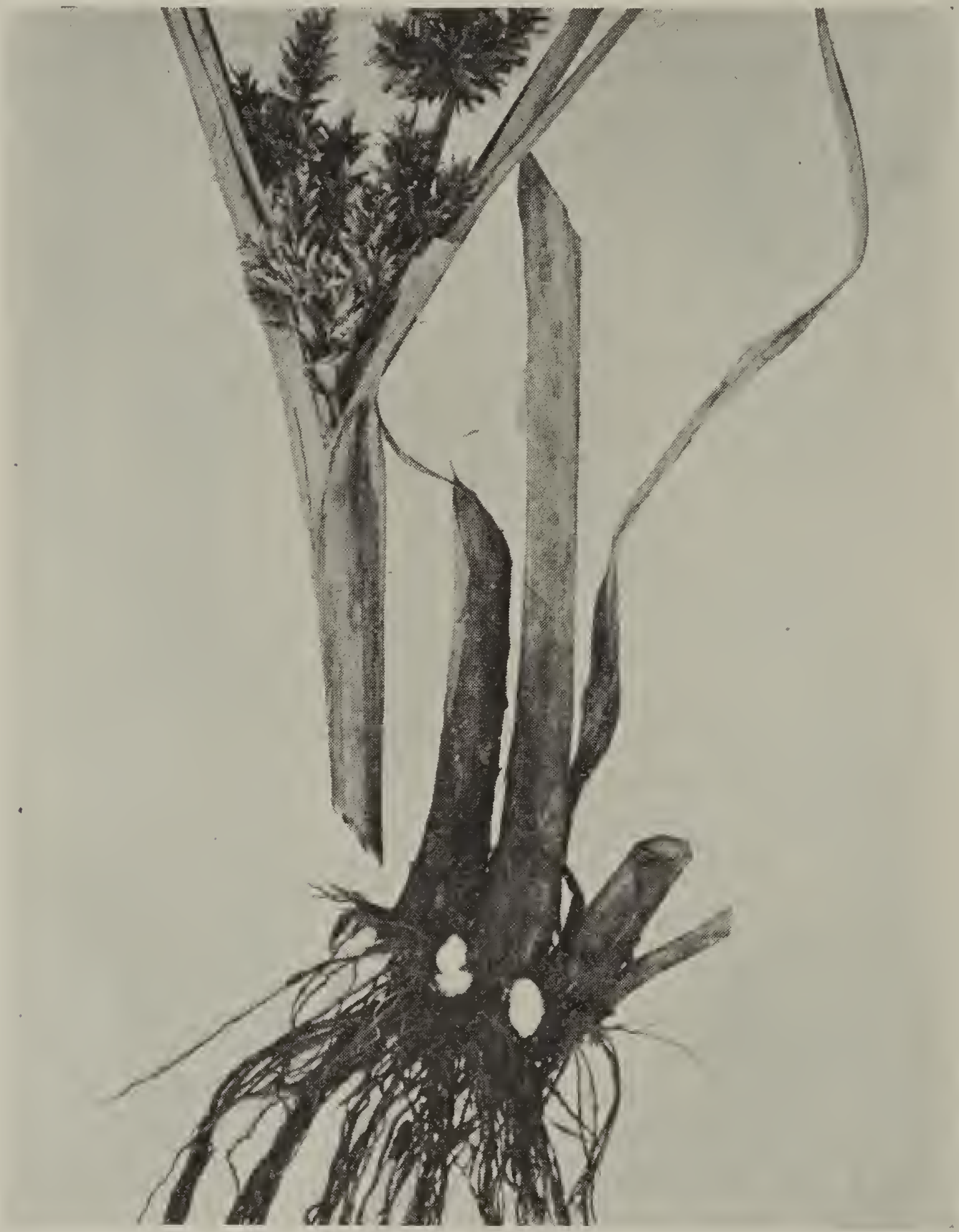


Figure 11. Southern Corn Bill Bug larvæ in crown of Red Rooted Cyperus Grass. X ½

LIFE HISTORY SUMMARY

The following summary is based largely upon observations made on the Pender Test Farm in the southeastern part of the State, but it is thought that the same conditions would be found practically throughout the area of greatest prevalence of the corn bill bug (Fig. 2). The adults hibernate over winter (Fig. 14), going into hibernation in late October (October 17th being the latest recorded date). They emerge from hibernation in mid-April (April 10th being the earliest date recorded). Their numbers seem to increase rather rapidly until late May and after that rather slowly until mid-August. This increase is perhaps largely due to the fact that new adults are continually emerging from pupa whose larva have developed from eggs laid early in the spring. After mid-August the numbers of adults diminish rapidly, so that in early corn practically all the adults have disappeared by the end of August. A few adults remain active to late October. The adults commence to lay eggs by early May (May 5th being the earliest recorded date). The number of eggs found in the field increases rather rapidly until mid-June and then less rapidly until mid-July, the number of eggs falling off slightly toward mid-August. After which the number decreases rather rapidly. The latest date recorded for finding eggs in the field is September 23d, but as they were still rather common at that time, it seems safe to conclude that egg laying continues until early October, especially as the adults are active in the fields till late October. The distribution of the larvæ throughout the year seems to coincide rather closely with the distribution of the eggs. The earliest larvæ have been found in the field in mid-May, but the time of greatest abundance seems to be from late July to mid-August. After this time their numbers fall off rapidly, the latest larvæ being found in late October. (Larvæ in what appeared to be the fourth molt being found on October 27th.) The earliest pupæ have been found in late June. The number increases rather rapidly till late July and seems to remain nearly constant till late September; the latest pupa being found on November 9th. These late maturing pupæ seem to all change to adults before winter, and these adults seem to remain in the

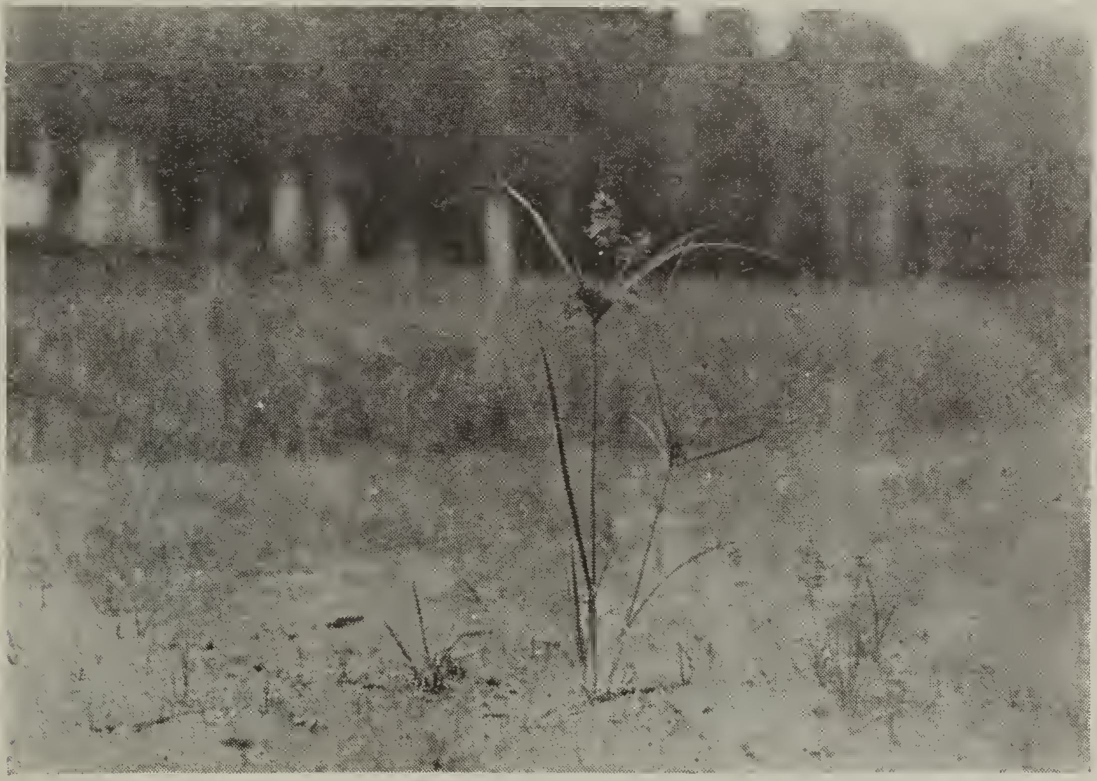


Figure 12. Cyperus Grass growing in a swamp to show characteristics.

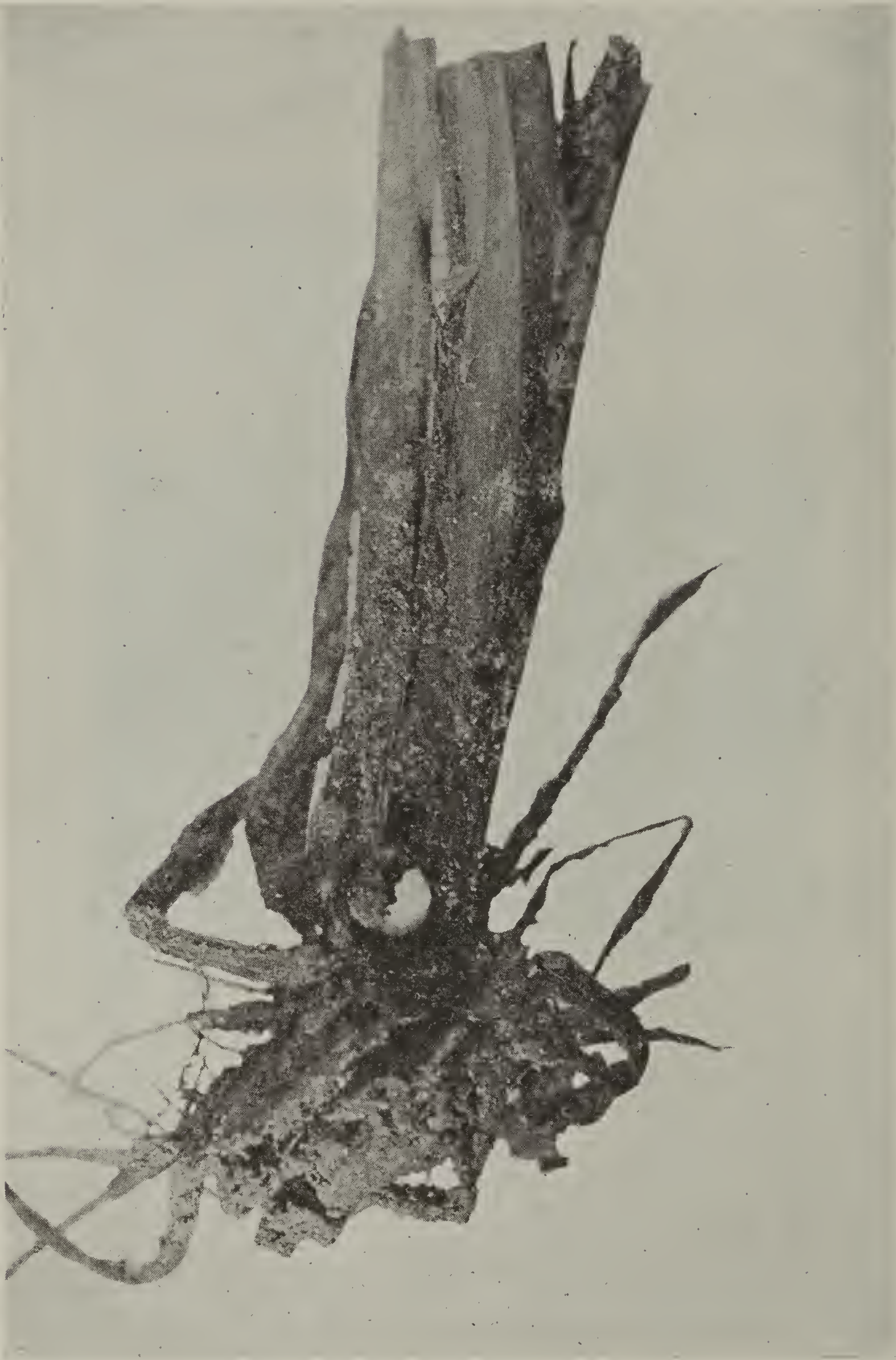


FIGURE 13. Larva feeding on the roots of Cyperus Grass.

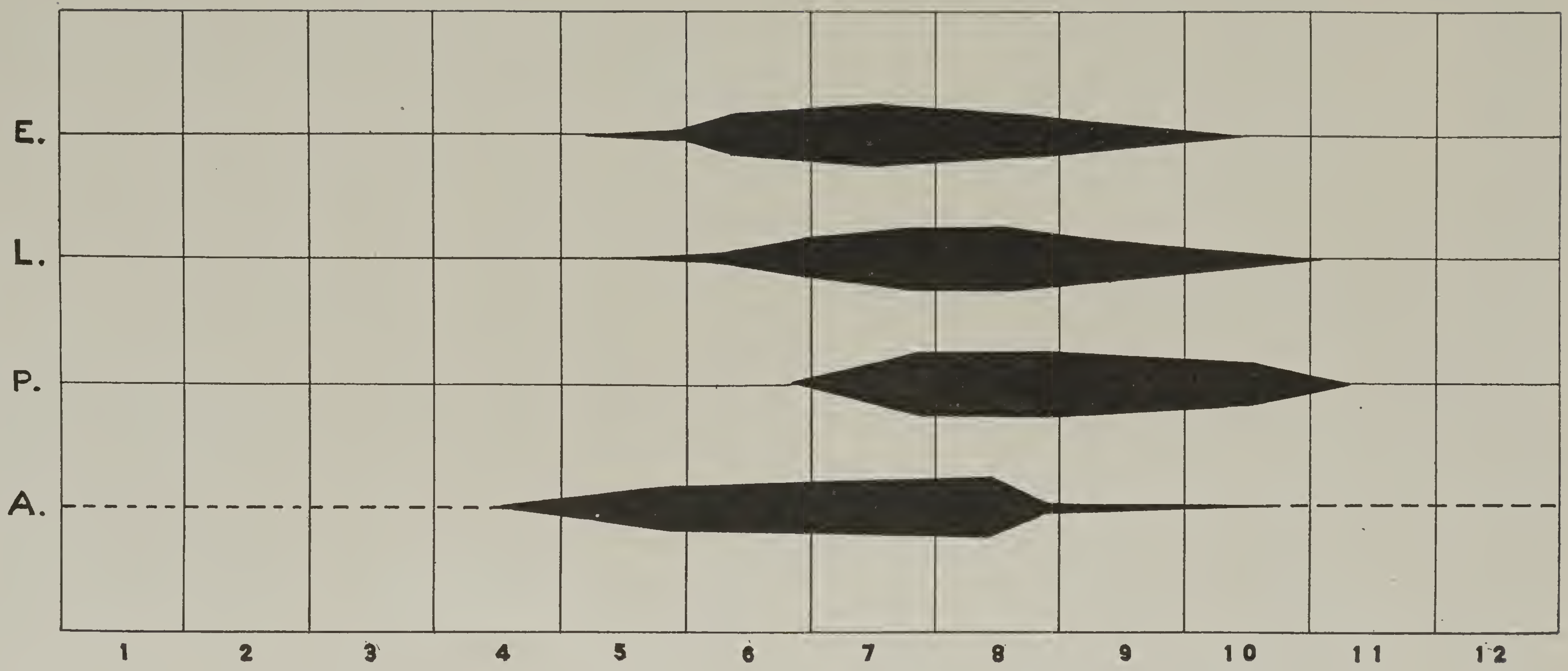


FIGURE 14. Life history summary. E. Eggs. L. Larvæ. P. Pupæ. A. Adults. The dotted line shows hibernation time. The figures at the bottom refer to the months.

pupal cells over winter, not becoming active until the following spring. There is some evidence to show that the early maturing adults lay eggs the same summer that they reach maturity, these eggs hatching and reaching maturity late in the season.

THE EGG DESCRIPTION

The egg is subreniform-elliptical in shape, almost pure white in color, and almost perfectly smooth (Fig. 15). As the egg grows older it becomes more yellowish. Its size varies greatly, as is indicated by the following table, which shows the lengths and greatest diameters of 51 eggs selected at random throughout the egg-laying season. The measurements were taken by means of a binocular microscope fitted with micrometer eyepiece calibrated to 0.0416 m.m. Inasmuch as these eggs were selected at random throughout the season, they probably represent fairly average conditions for North Carolina. No constant differences between the sizes of eggs taken early in the season and those taken later in the season can be detected from the measurements.

MEASUREMENTS OF 51 EGGS OF THE SOUTHERN CORN BILL BUG—MEASUREMENTS TAKEN AT VARIOUS TIMES THROUGHOUT THE SEASON.

TABLE I.

Egg Number	Total Length, Measured as a Straight Line	Greatest Diameter
1	2.08 mm.	.95 mm.
2	2.91 mm.	1.03 mm.
3	2.08 mm.	1.03 mm.
4	2.36 mm.	1.03 mm.
5	2.95 mm.	.91 mm.
6	2.91 mm.	.91 mm.
7	2.50 mm.	.95 mm.
8	2.41 mm.	.95 mm.
9	2.33 mm.	1.03 mm.
10	2.54 mm.	.91 mm.
11	2.37 mm.	.86 mm.
12	2.50 mm.	.86 mm.
13	2.08 mm.	.95 mm.
14	2.28 mm.	.83 mm.
15	2.33 mm.	.83 mm.
16	2.45 mm.	.86 mm.
17	2.28 mm.	.86 mm.
18	2.95 mm.	.86 mm.
19	2.28 mm.	.91 mm.
20	2.24 mm.	.86 mm.
21	2.37 mm.	.99 mm.
22	2.50 mm.	.95 mm.
23	2.50 mm.	.99 mm.
24	2.24 mm.	.91 mm.
25	1.95 mm.	.98 mm.
26	2.41 mm.	.98 mm.
27	2.17 mm.	.70 mm.
28	2.46 mm.	.96 mm.
29	2.02 mm.	.88 mm.

Egg Number	Total Length, Measured as a Straight Line	Greatest Diameter
30	2.15 mm.	.90 mm.
31	2.54 mm.	.96 mm.
32	2.10 mm.	.93 mm.
33	2.21 mm.	.91 mm.
34	2.12 mm.	.91 mm.
35	2.19 mm.	.89 mm.
36	2.12 mm.	.91 mm.
37	2.05 mm.	.93 mm.
38	2.21 mm.	.92 mm.
39	2.28 mm.	.88 mm.
40	2.10 mm.	.88 mm.
41	2.19 mm.	.91 mm.
42	2.31 mm.	.91 mm.
43	2.57 mm.	.93 mm.
44	2.29 mm.	.85 mm.
45	2.27 mm.	.95 mm.
46	2.41 mm.	.91 mm.
47	2.18 mm.	.89 mm.
48	2.29 mm.	.87 mm.
49	2.54 mm.	.93 mm.
50	2.39 mm.	1.04 mm.
51	2.42 mm.	.93 mm.

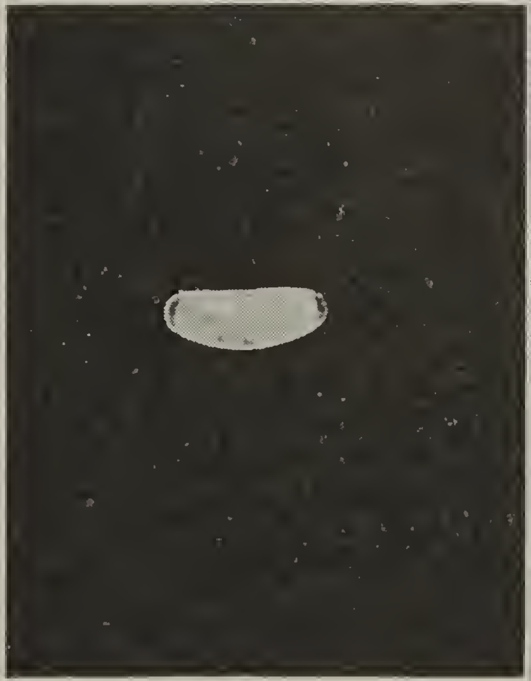
THE EGG. TIME OF HATCHING

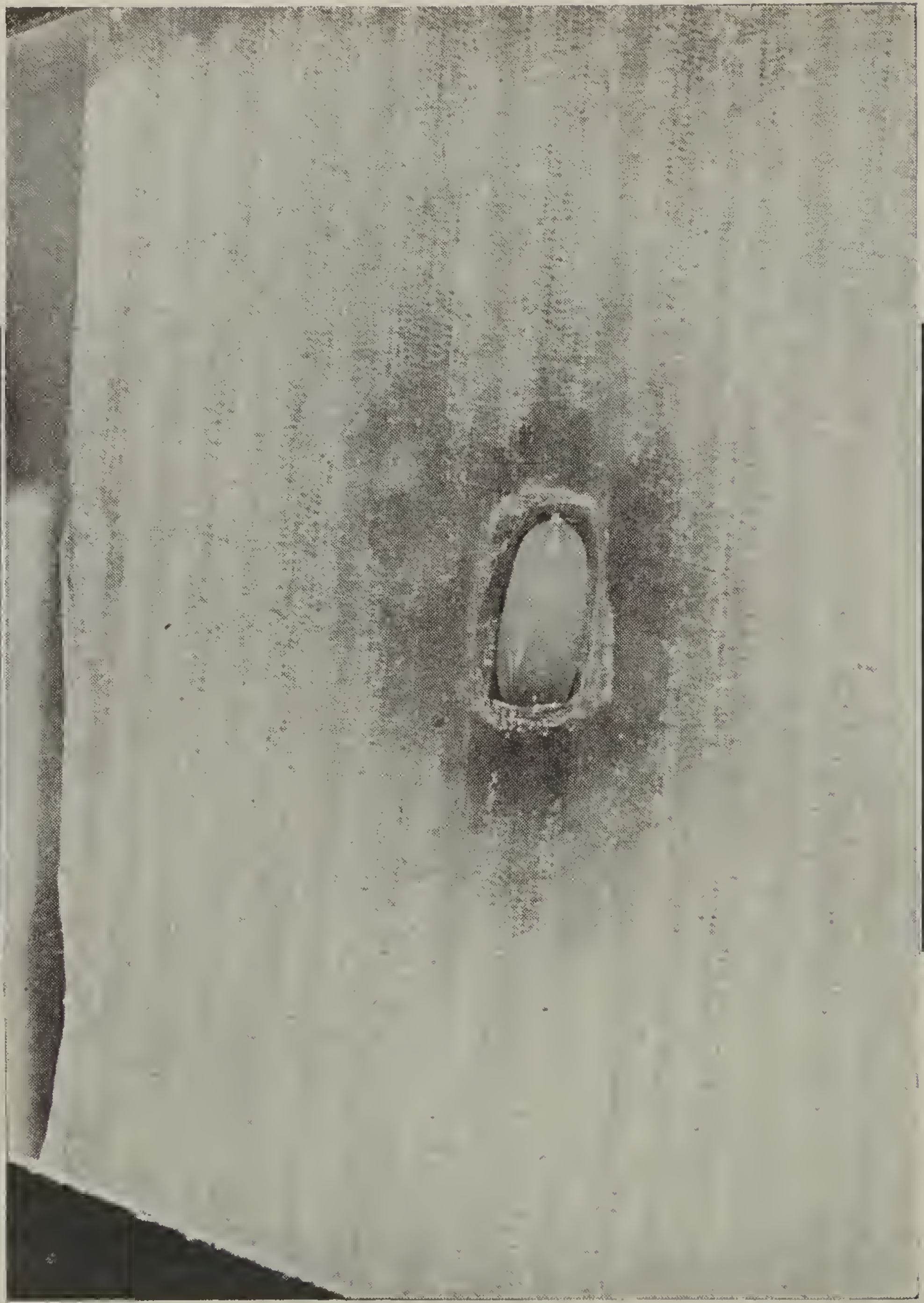
During the egg-laying period records have been kept of a total of 143 separate lots of eggs containing a total of 903 eggs. These eggs were hatched under varying conditions both as to temperature and moisture. Some of the eggs were hatched in pieces of cornstalks placed in vials or in tin boxes, and in some cases where there were large numbers of eggs they were placed in tin boxes together with the piece of corn on which they were laid. In some cases the records were secured to within four hours, in other cases to within not more than twenty-four hours.

These tables will show that there is great variation in the time of hatching of the eggs of the Southern Corn Bill Bug, and while the writer believes that this variation can be controlled to a very great extent by giving identical conditions, he also believes that no such uniform conditions exist in nature. Therefore, it seems perfectly safe to conclude that the eggs of the Southern Corn Bill Bug hatch in not less than 77 hours and that the time may be greatly extended certainly to 305 hours (Fig. 18), the mean time being 132 hours.

The figures in some of the tables are very accurate. This is especially true of the figures in Table II, where some of the figures are to within less than four hours.

In securing the figures for Tables III and IV the usual practice was to give the adults fresh food about 6 o'clock in the evening and then to remove this food about 8 o'clock in the morning. In most of the cases the eggs were examined about 8 o'clock in the morning, at noon, and





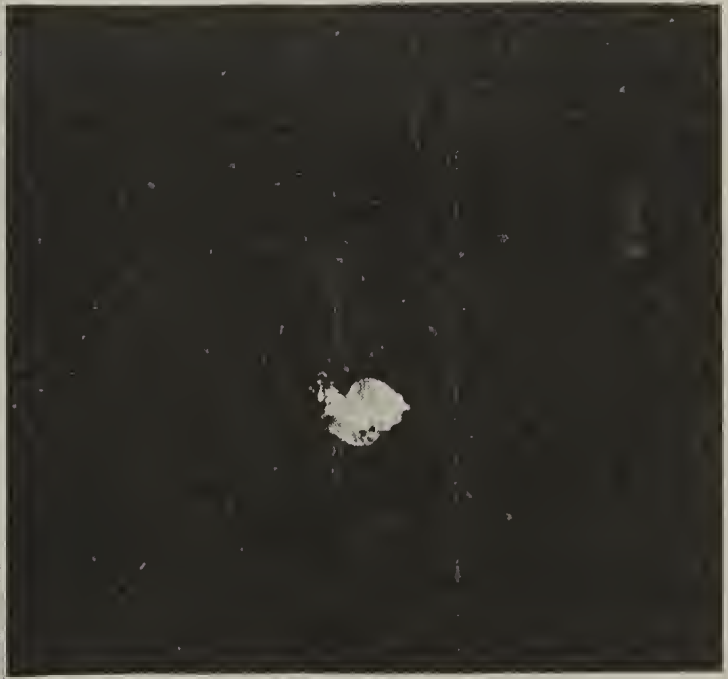


FIGURE 15. Egg of Southern Corn Bill Bug. X6. FIGURE 16. Egg of Southern Corn Bill Bug in natural position in pocket in outer leaf blade. X9. FIGURE 17. Larva of Southern Corn Bill Bug. Just hatched. X6.

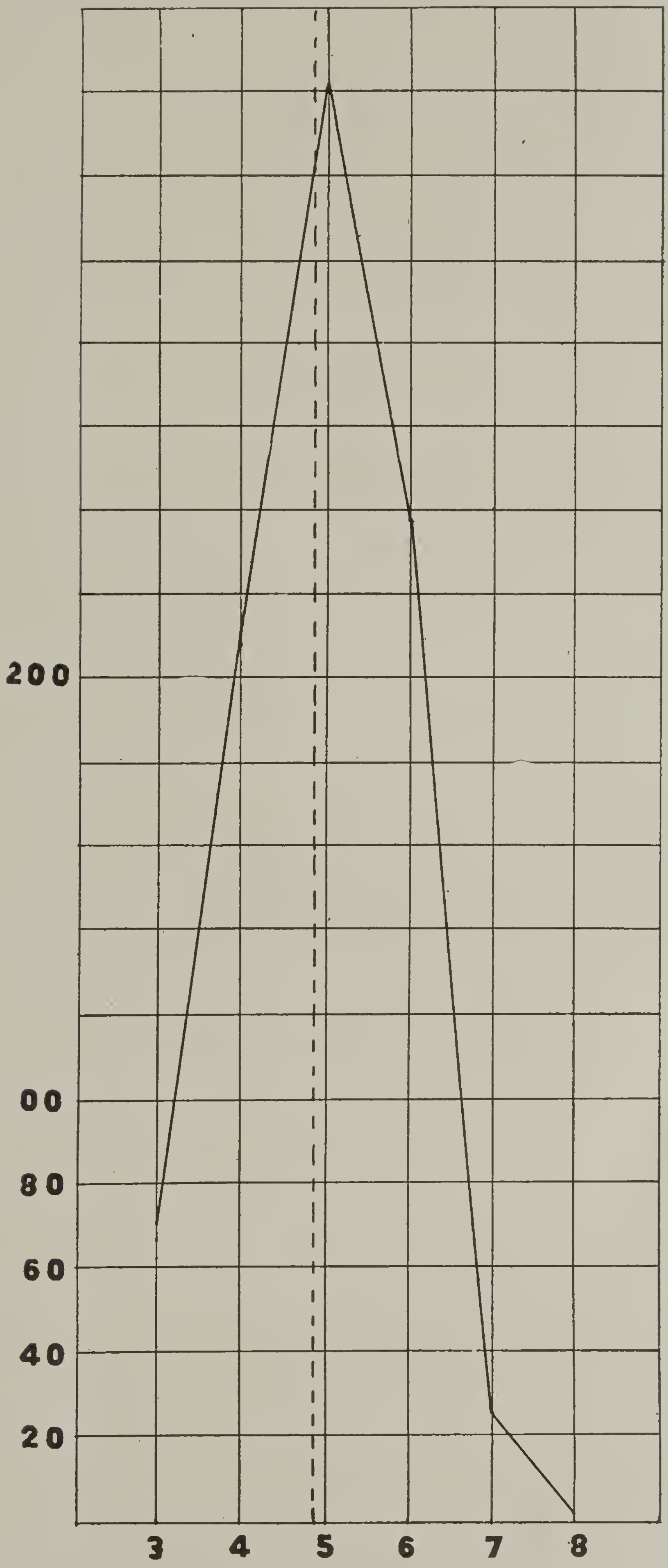


FIGURE 18. Curve to show time of hatching for 891 eggs of the Southern Corn Bill Bug expressed in one-day intervals. The mean time is shown by the dotted vertical line (........).

again in the evening. In most of the cases, as will be seen from the above figures, the eggs hatched about noon on the fourth, fifth, sixth, and seventh days following the night on which they were laid.

Frequently eggs laid the same night in the same piece of corn and stored in the same tin box under what must obviously be the same conditions hatched at varying times from 102 to 174 hours.

This point was made a special issue for field experiment during the summer of 1914, and while no definite data could be secured, owing to the fact that the eggs were buried in the stalk where they could not be observed, yet females were watched ovipositing and the egg marked as carefully as possible and then examined three, four, five, six, and seven days later. Most of these eggs were laid in the morning between 5 and 8 o'clock and most of them were examined in the evening between 4 and 6; hence our results are not much closer than twelve hours. But out of twenty-three eggs examined in this way, fairly close time was secured on only six, the shortest observed time was 130 hours and the longest recorded time was 177 hours. The other four eggs that had hatched when examined and near which young larvæ were discovered were found in the evening of the sixth day after they were laid, giving a total time of about 154 hours from the time they were laid.

These figures agree very well with the figures as from the following tables.

TABLE II.

INCUBATION RECORD OF 53 LOTS OF EGGS HATCHED IN THE INSECTARY DURING THE SUMMER OF 1911.

Lot No.	No. of Eggs	Number of Hours Required for Hatching		
		Minimum	Maximum	Average
1	6	151	161	156
2	1	150	160	155
3	2	156	160	158
4	4	168	178	173
5	1	174	184	179
6	1	188	195	192
7	1	193	205	199
8	2	170	183	177
9	1	170	183	177
10	1	158	169	164
11	1	150	166	158
12	5	123	147	135
13	5	141	165	153
14	4	127	151	139
15	1	186	192	189
16	26	148	164	156
17	5	139	143	141
18	1	305	329	317
19	4	137	141	139
20	3	161	165	163
21	1	141	142	142
22	3	161	163	162

Lot No.	No. of Eggs	Number of Hours Required for Hatching		
		Minimum	Maximum	Average
23	5	144	159	152
24	1	137	139	138
25	2	148	150	149
26	5	132	147	140
27	6	145	160	153
28	12	145	165	155
29	1	162	164	163
30	3	120	144	132
31	1	144	168	151
32	1	159	161	160
33	8	146	167	151
34	1	129	150	140
35	1	210	216	218
36	2	159	164	162
37	5	223	247	135
38	10	148	172	160
39	5	144	168	156
40	6	141	173	156
41	4	128	147	138
42	4	142	165	154
43	23	147	158	153
44	3	132	142	137
45	7	141	151	146
46	2	144	160	152
47	10	128	144	136
48	8	151	172	162
49	6	137	144	141
50	22	136	143	140
51	11	139	143	141
52	13	96	120	108
53	3	94	118	106

TABLE III

INCUBATION RECORD OF 25 LOTS OF EGGS HATCHED IN THE INSECTARY DURING THE SUMMER OF 1913.

1	4	103	115	109
2	5	124	136	130
3	11	102	114	108
4	5	108	120	114
5	5	84	96	90
6	15	103	115	109
7	4	77	89	83
8	6	84	96	90
9	6	102	118	108
10	6	78	90	84
11	8	109	121	115
12	17	85	97	91
13	7	109	121	115
14	10	80	92	86
15	4	85	97	91
16	3	102	114	108
17	4	132	144	138
18	13	133	145	139

Lot No.	No. of Eggs	Number of Hours Required for Hatching		
		Minimum	Maximum	Average
19	8	134	146	140
20	15	158	170	164
21	6	120	132	126
22	16	133	145	139
23	16	109	121	115
24	21	109	121	115
25	19	84	96	90

TABLE IV

INCUBATION RECORDS OF 65 LOTS OF EGGS HATCHED IN THE LABORATORY DURING THE SEASON OF 1915.

1	7	102	114	108
2	8	126	138	132
3	8	102	114	108
4	9	127	139	133
5	8	103	115	109
6	10	133	145	139
7	11	109	121	115
8	2	128	140	134
9	2	104	116	110
10	7	126	138	132
11	5	102	114	108
12	10	125	137	133
13	6	126	133	132
14	7	150	162	156
15	4	174	186	180
16	11	150	162	156
17	4	174	186	180
18	6	126	138	132
19	6	150	162	156
20	2	174	186	180
21	6	126	138	132
22	3	150	162	156
23	1	174	186	180
24	7	126	138	132
25	2	150	162	156
26	3	174	186	180
27	10	126	138	132
28	2	150	162	156
29	6	150	162	156
30	3	174	186	180
31	2	126	138	132
32	6	150	162	156
33	13	126	138	132
34	2	150	162	156
35	14	102	114	108
36	5	126	138	132
37	13	102	114	108
38	4	126	138	132
39	15	102	114	108
40	3	126	138	132
41	1	102	114	108
42	15	126	138	132

Lot No.	No. of Eggs	Number of Hours Required for Hatching		
		Minimum	Maximum	Average
43	6	102	114	108
44	6	126	138	132
45	3	102	114	108
46	4	126	138	132
47	2	102	114	108
48	8	126	138	132
49	1	102	114	108
50	11	126	138	132
51	12	126	138	132
52	10	126	138	132
53	5	126	138	132
54	5	150	162	156
55	8	150	162	156
56	5	126	138	132
57	4	150	162	156
58	7	150	162	156
59	8	102	114	108
60	3	126	138	132
61	7	102	114	108
62	4	126	138	132
63	6	126	138	132
64	3	150	162	156
65	2	126	138	132

WHERE LAID

Practically all of the eggs laid in the field are placed in the base of the cornstalk below the surface of the ground, or within an inch above the surface of the ground. In the cyperus plant the female seems to have a tendency to lay the eggs higher up. In young corn the eggs are usually laid deep in the heart of the stalk in especially prepared cavities (Fig. 8), but in older stalks the eggs are more frequently laid in the sheath of the outer blade, in a little pocket (Fig. 16).

Frequently in the field the eggs are loosely placed below the surface of the ground among the roots. In all cases where females had been watched closely they seemed to have a tendency to lay the last egg carelessly. In several cases where females had been observed to deposit one egg with the greatest care, the second egg was deposited in only a very shallow cavity or loosely among the roots. These females gave every evidence of being tired of the process of oviposition, and seemed to abandon all their usual precautions in egg laying.

Frequently, also, in our breeding cages loose eggs would be deposited. This tendency seemed to be greater when a layer of sand was placed in the breeding cages. So far as observed, these eggs seemed to be as fertile as eggs more carefully deposited. In no case where the female deposited only a single egg was this egg deposited loosely, but always, so far as the writer's observations go, such eggs were deposited with the greatest care.

NUMBER LAID

The number of eggs laid by single females was the subject of a detailed experiment by Mr. Smith. The total number of eggs laid each day by 35 pairs as recorded by him is reproduced below in Table V. These records run from May 24th to September 29th. The daily egg-laying record of 23 pairs as recorded by the writer during the summer of 1914 is appended as Table VII. These records run from June 16th to August 24th.

In securing these records, single pairs were isolated in small jelly glasses and provided with a short section of cornstalk. These sections of cornstalk were cut from fresh stalks secured from the field every day, the sections being cut from the base of the stalk near the ground. In as far as possible, an attempt was made to secure food comparable to that found in the field. Towards the end of the season these stalks became hard and unsatisfactory for food, but they were always comparable to stalks found in the field.

In the field our records show that egg laying commences in early May, May 5th being the earliest date recorded at Willard. Egg laying continues through the season until September 21st in the field. But after August 15th the number of eggs diminishes very rapidly, owing perhaps to the fact that suitable corn cannot usually be found after that date. There is some evidence that the adults emigrate to cyperus from the cornfields late in the season, but the writer has never found eggs on cyperus after the middle of August. From these records it would seem that egg laying in the field actually surpasses our longest insectary record. This is perhaps due to the fact that insects brought from the eastern part of the State are set back in their egg laying, due, perhaps, to differences of temperature and moisture, and perhaps due to unnatural conditions during transportation. In part, the larger field record might be due to young adults which emerge in midsummer and lay a few eggs, but the fact that some of the adults in the insectary laid eggs as late as the 29th of September, at least a week later than eggs have been found in the field, would tend to minimize this factor.

According to our records, the greatest number of eggs laid in any single day was 11, and records of 5 to 10 are fairly common. The greatest number of eggs laid by any female, so far as observed, in the field, under perfectly natural conditions, was three eggs between 4:30 a. m. and 9:15 a. m. and then again in the evening between 6:30 and 7 she laid a single egg. In all, during the season of 1912 470 individuals were marked and observed for their egg-laying habits. None of these females were observed to lay eggs every day, but they were observed to lay often enough to lead one to conclude that they did lay every day. Many individuals were observed to lay a single egg, and in one case one female was observed to lay an egg each day for three days in succession. Other individuals were observed to lay eggs on many separate occasions. Four individuals were observed to lay two eggs in the same day, these individuals being under almost continuous observation from 3:30 a. m. to 11 p. m.

From these observations it would seem safe to conclude that our daily egg records taken in the insectary would not be far from the conditions as they exist in the field; that is, that each female normally lays from one to at least four and perhaps more eggs each day from early May to late August, and perhaps more or less regularly from late August to late September.

TABLE V

EGG LAYING RECORDS

1911		Pair Number											
		1	2	3	4	5	6	7	8	9	10	11	12
May	24	------	4	------	------	------	------	------	------	------	------	------	------
	25	------	1	------	------	------	------	------	------	------	------	------	------
	26	------	1	------	------	------	------	------	------	------	------	------	------
	27	------	1	------	------	------	------	------	------	------	------	------	------
	28	3	3	0	5	0	2	5	5	1	1	5	5
	29	2	0	4	1	5	4	2	3	1	4	2	1
	30	2	2	0	1	1	1	3	2	0	1	0	4
	31	4	3	1	3	4	4	4	3	2	1	0	3
June	1	3	3	3	2	5	3	4	3	0	1	1	4
	2	4	4	3	4	7	3	4	4	1	1	4	4
	3	4	4	1	0	3	4	3	2	3	3	3	5
	4	1	4	4	3	6	6	3	5	0	3	4	3
	5	1	0	2	0	4	4	1	3	2	1	2	6
	6	3	5	5	0	6	3	3	1	0	4	7	5
	7	2	3	1	1	6	5	4	3	1	3	4	3
	8	1	3	2	0	3	4	1	2	0	0	4	5
	9	2	2	0	0	4	4	2	3	0	3	3	3
	10	1	3	3	0	3	3	1	0	2	4	3	6
	11	2	2	1	1	4	3	3	3	0	2	2	4
	12	4	3	3	1	9	3	2	2	2	5	5	6
	13	3	3	0	1	3	1	1	2	2	2	5	3
	14	3	4	3	3	6	0	1	1	1	0	3	7
	15	2	3	3	0	4	5	4	1	0	2	4	2
	16	1	4	4	2	7	6	0	2	1	4	5	8
	17	4	1	2	2	3	2	5	2	1	4	2	7
	18	2	3	2	0	4	4	1	2	1	1	6	1
	19	4	2	4	3	4	6	4	3	3	4	4	4
	20	2	3	2	0	5	5	0	2	2	3	5	4
	21	2	2	3	2	6	5	3	2	3	4	4	5
	22	2	4	2	1	5	3	1	2	2	2	2	4
	23	2	5	3	2	3	4	3	1	3	4	5	8
	24	3	3	4	2	5	7	2	4	3	4	5	1
	25	3	4	3	1	8	3	2	3	1	2	4	6
	26	4	4	4	2	6	7	2	3	2	3	6	5
	27	4	5	3	0	3	8	1	1	0	1	6	9
	28	2	6	3	3	6	5	0	4	3	1	3	4
	29	3	6	5	0	4	4	0	3	2	0	6	6
	30	2	4	5	1	2	6	2	2	3	0	5	2
July	1	2	4	6	2	4	5	0	2	2	0	2	4
	2	0	5	3	1	2	7	0	2	4	0	3	11
	3	1	5	6	0	3	5	2	1	2	0	6	5
	4	1	4	4	0	4	5	0	5	2	0	4	4
	5	0	3	2	1	4	3	1	4	2	0	4	6
	6	0	6	4	1	4	5	0	3	4	0	4	4
	7	1	3	2	0	3	3	1	3	1	0	7	8
	8	1	7	3	0	5	2	0	5	3	0	3	5
	9	2	5	3	0	4	5	0	2	3	0	5	5
	10	3	4	3	0	3	3	2	4	3	0	4	4
	11	1	6	3	0	1	8	0	2	1	0	3	4
	12	0	5	4	0	4	5	1	4	3	0	4	9
	13	2	6	1	0	5	1	1	4	3	0	4	9
	14	3	5	2	0	4	5	0	2	3	0	4	4
	15	3	3	2	0	2	3	0	2	2	0	5	5
	16	0	5	3	0	4	5	1	4	5	0	3	7
	17	0	5	3	0	6	4	0	3	3	0	6	6

Table V.

FOR 35 PAIRS, SUMMER 1911.

Pair Number

13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	1	----	----
----	----	----	1	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	2	----	----
----	----	----	0	----	1	----	----	----	----	----	1	----	----	0	2	----	----	----	----	0	----	----
----	----	----	0	----	1	----	----	----	----	----	0	----	3	2	1	----	----	----	----	1	----	----
4	2	0	1	4	0	5	2	4	1	4	0	1	2	0	0	2	0	0	5	0	0	0
0	3	0	1	3	1	2	1	2	3	2	1	1	2	0	1	3	0	0	3	0	3	0
2	1	0	0	4	5	2	0	0	0	1	2	3	2	0	1	0	0	0	0	0	0	0
0	2	9	2	4	0	3	2	7	3	3	0	2	2	1	1	0	6	0	1	0	3	0
3	2	4	1	4	2	4	2	3	1	2	1	1	2	0	3	2	0	3	2	0	0	0
1	3	3	2	2	6	3	2	9	2	4	3	1	2	0	0	0	0	0	4	0	0	0
2	1	3	0	3	2	3	1	4	1	1	1	0	2	0	1	0	2	0	2	0	0	1
2	0	1	1	2	2	2	2	4	3	3	1	2	1	0	0	0	1	1	3	0	1	0
1	1	4	1	5	1	1	0	1	3	5	0	1	2	0	1	0	0	0	1	0	1	0
3	2	5	1	2	5	4	0	8	2	1	1	0	3	0	0	1	0	2	1	0	0	0
1	2	2	4	2	1	0	1	4	2	1	0	0	0	0	1	0	0	0	0	0	0	1
0	0	2	1	1	3	3	2	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0
0	2	4	1	1	0	0	0	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0
2	2	4	1	0	5	3	1	6	3	1	0	0	0	0	0	0	1	0	0	0	0	0
0	1	2	0	1	1	1	0	1	3	3	0	0	0	0	0	0	0	0	0	0	0	0
2	5	6	1	2	3	2	2	3	3	1	1	1	0	0	0	0	0	0	0	0	0	0
1	3	5	1	2	3	3	1	1	3	2	1	0	0	0	0	1	0	0	1	0	0	0
0	0	6	4	1	2	1	1	6	4	3	0	0	0	0	0	0	0	0	1	0	0	0
1	1	4	1	0	2	1	0	1	2	3	0	0	0	0	0	0	0	0	0	0	0	1
1	6	8	2	2	3	0	2	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0
0	2	7	0	1	5	0	0	5	3	3	0	0	0	0	0	0	0	0	0	0	0	1
0	0	2	1	1	2	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	4	7	0	1	5	0	0	3	3	2	1	0	0	1	0	0	0	0	0	0	0	0
0	2	6	2	0	2	1	1	4	0	3	1	0	0	0	0	0	0	0	0	0	0	0
1	1	4	0	2	3	0	2	4	6	1	0	0	0	0	0	0	0	0	0	0	0	0
2	3	5	0	0	4	1	0	3	2	3	0	0	0	0	0	0	0	2	0	0	0	0
2	1	5	0	2	4	0	0	2	5	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	10	0	1	4	0	0	1	4	4	0	0	0	0	0	0	0	0	0	0	0	0
1	3	5	0	4	2	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0
2	4	9	0	3	5	0	0	3	4	1	0	0	0	0	0	0	0	0	0	0	0	0
1	3	7	0	0	5	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	5	0	3	2	0	0	2	3	0	1	0	0	0	0	0	0	0	0	0	0	0
0	3	7	0	0	2	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	6	0	2	4	2	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0
1	3	6	0	0	3	0	0	4	5	1	1	0	0	0	0	0	0	0	0	0	0	0
0	2	5	0	0	3	0	0	2	4	2	0	0	0	0	0	0	0	0	0	0	0	0
0	3	4	0	1	2	0	0	3	4	2	0	0	0	0	5	0	0	4	0	0	0	4
1	2	4	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	2	7	0	0	2	0	0	4	5	1	0	0	0	1	0	0	0	0	0	0	0	0
0	5	5	0	1	1	1	0	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0
0	4	0	0	0	0	0	0	3	4	1	0	0	0	0	0	0	0	0	0	0	0	0
0	4	4	D	0	2	0	0	2	8	0	0	0	0	0	0	0	0	0	0	0	0	0
0	4	2	----	0	1	0	0	3	4	3	2	0	0	0	0	0	0	0	0	0	0	0
0	3	6	----	1	1	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0
0	5	3	----	0	0	3	0	5	6	2	0	0	0	0	0	0	0	0	0	0	0	0
0	5	7	----	0	0	4	1	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0
0	3	6	----	0	1	4	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
0	4	6	----	0	0	5	0	2	5	1	1	0	0	0	0	0	0	0	0	0	0	0
0	4	3	----	0	0	5	0	2	5	2	0	0	0	0	0	0	0	0	0	0	0	0
0	3	3	----	0	0	6	0	3	5	2	0	0	0	0	0	0	0	0	0	0	0	0
0	2	3	----	2	1	4	0	3	4	1	1	0	0	0	0	0	0	0	0	0	0	0

1911		Pair Number											
		1	2	3	4	5	6	7	8	9	10	11	12
July	18	1	7	4	0	3	3	0	6	4	0	4	6
	19	1	5	6	0	3	4	1	4	3	0	3	5
	20	0	2	7	0	2	7	0	4	3	0	1	3
	21	1	7	5	1	5	5	1	3	5	0	5	8
	22	0	4	8	0	2	7	2	2	4	0	6	2
	23	0	3	4	0	5	4	1	3	4	0	4	5
	24	1	6	5	0	3	2	1	5	5	0	6	4
	25	1	7	10	0	5	10	2	2	5	0	4	7
	26	0	1	3	0	1	3	1	1	3	0	5	2
	27-28	1	7	9	0	5	6	2	5	6	0	9	6
	29	0	7	7	0	4	5	2	2	3	0	5	1
	30	1	6	3	0	3	7	3	3	5	0	5	6
	31	1	3	4	0	3	2	3	3		0	3	3
August	1	1	8	6	0	6	8	1	0	5	0	6	2
	2	1	3	2	0	3	4	2	2	2	0	3	3
	3	1	5	4	0	3	1	0	2	4	0	7	0
	4	1	5	7	0	4	5	1	0	4	0	6	0
	5	0	6	3	0	3	4	1	2	2	0	4	0
	6	2	4	7	0	5	4	1	0	6	0	6	0
	7	0	4	7	0	4	4	0	0	2	0	8	0
	8-9-10	0	11	9	0	5	10	0	0	11	0	12	2
	11	0	4	6	0	1	4	0	0	3	0	6	0
	12	0	5	6	0	1	4	0	0	2	0	4	0
	13-14-15	0	13	8	0	5	11	0	0	4	0	10	2
	16	0	5	2	0	0	0	0	0	4	0	2	------
	17	0	2	1	0	0	2	0	0	1	0	0	------
	18	0	8	3	0	1	3	0	0	5	0	3	------
	19	0	3	3	0	4	6	0	0	4	0	4	------
	20	0	5	3	0	1	3	0	0	3	0	4	------
	21	0	0	0	0	0	0	0	0	0	0	0	------
	22	0	10	2	0	4	6	0	0	5	0	5	------
	23	0	0	0	0	0	0	0	0	0	0	0	------
	24	0	4	4	0	1	4	0	0	1	0	5	------
	25-26	0	5	0	0	0	7	0	0	1	0	7	------
	27	0	4	0	0	2	2	0	0	3	0	6	------
	28	0	4	0	0	1	2	0	0	4	0	2	------
	29	0	4	0	0	1	5	0	0	4	0	6	------
	30	0	5	0	0	2	5	0	0	3	0	2	------
	31	0	5	0	0	0	4	0	0	3	0	1	------
September	1-2	0	7	1	0	4	5	0	0	5	0	6	------
	3-4	0	7	7	0	3	4	0	0	6	0	6	------
	5	0	2	2	0	0	1	0	0	1	0	3	------
	6	0	5	0	0	0	5	0	0	2	0	0	------
	7	0	2	0	0	0	0	0	0	4	0	0	------
	8	0	3	0	0	0	1	0	0	2	0	0	------
	9	0	5	0	0	0	1	0	0	1	0	4	------
	10-11	0	7	0	0	1	3	0	0	1	0	2	------
	12	0	4	0	0	0	0	0	0	0	0	2	------
	13	0	1	0	0	1	0	0	0	0	0	4	------
	14	0	0	0	0	0	1	0	0	0	0	1	------
	15	0	1	0	0	0	1	0	0	0	0	0	------
	16	0	2	3	0	1	2	0	0	0	0	0	------
	17	0	0	0	0	0	0	0	0	0	0	0	------
	18	0	3	0	0	0	1	0	0	0	0	0	------
	19	0	1	0	0	0	1	0	0	0	0	1	------

Pair Number																						
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	3	8	----	1	0	5	0	4	3	1	2	0	0	0	0	0	0	0	0	0	0	0
0	4	3	----	0	0	6	0	3	4	3	0	0	0	5	4	0	0	0	0	0	0	0
0	2	4	----	0	0	4	0	2	3	2	2	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.
0	5	4	----	0	0	4	0	3	7	1	1	----	----	----	----	----	----	----	----	----	----	----
0	4	1	----	1	1	5	0	3	4	1	1	----	----	----	----	----	----	----	----	----	----	----
0	3	3	----	0	0	5	0	2	7	1	1	----	----	----	----	----	----	----	----	----	----	----
0	5	1	----	0	0	4	0	2	5	1	0	----	----	----	----	----	----	----	----	----	----	----
0	5	5	----	0	1	6	0	2	6	1	0	----	----	----	----	----	----	----	----	----	----	----
0	2	0	----	0	0	1	0	4	1	1	1	----	----	----	----	----	----	----	----	----	----	----
0	8	5	----	1	1	3	0	3	7	2	1	----	----	----	----	----	----	----	----	----	----	----
0	2	6	----	0	1	1	0	3	3	2	0	----	----	----	----	----	----	----	----	----	----	----
1	3	0	----	0	0	2	0	4	4	0	2	----	----	----	----	----	----	----	----	----	----	----
0	5	2	----	0	0	2	0	2	4	4	0	----	----	----	----	----	----	----	----	----	----	----
0	1	4	----	0	0	2	0	5	3	1	0	----	----	----	----	----	----	----	----	----	----	----
1	5	0	----	0	0	1	0	3	3	3	1	----	----	----	----	----	----	----	----	----	----	----
1	4	0	----	0	0	3	0	5	8	4	0	----	----	----	----	----	----	----	----	----	----	----
0	2	2	----	0	0	3	0	2	3	3	0	----	----	----	----	----	----	----	----	----	----	----
2	0	2	----	0	0	4	0	4	6	2	1	----	----	----	----	----	----	----	----	----	----	----
0	0	2	----	0	0	3	0	4	4	3	0	----	----	----	----	----	----	----	----	----	----	----
0	5	7	----	0	0	4	0	4	4	3	0	----	----	----	----	----	----	----	----	----	----	----
4	0	0	----	0	0	3	0	7	10	4	0	----	----	----	----	----	----	----	----	----	----	----
1	4	1	----	0	0	4	0	4	3	0	0	----	----	----	----	----	----	----	----	----	----	----
2	0	0	----	0	0	1	0	3	4	4	0	----	----	----	----	----	----	----	----	----	----	----
2	6	0	----	0	0	1	0	6	11	4	1	----	----	----	----	----	----	----	----	----	----	----
0	0	0	----	0	0	4	0	4	5	1	0	----	----	----	----	----	----	----	----	----	----	----
1	0	1	----	0	0	0	0	2	3	1	0	----	----	----	----	----	----	----	----	----	----	----
2	0	D	----	0	0	0	0	1	4	3	0	----	----	----	----	----	----	----	----	----	----	----
1	1	----	----	0	0	3	0	6	7	2	0	----	----	----	----	----	----	----	----	----	----	----
0	1	----	----	0	0	1	0	5	3	2	0	----	----	----	----	----	----	----	----	----	----	----
0	----	----	----	0	0	0	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
2	3	----	----	0	0	0	0	4	6	6	3	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	2	0	3	3	3	0	----	----	----	----	----	----	----	----	----	----	----
1	3	----	----	0	0	0	0	4	3	2	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	4	3	4	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	1	3	5	1	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	4	1	3	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	2	5	2	0	----	----	----	----	----	----	----	----	----	----	----
1	0	----	----	0	0	0	0	3	3	2	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	2	3	5	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	3	6	5	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	0	0	1	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	1	1	1	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	0	3	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	1	2	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	0	2	2	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	0	2	4	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	0	2	2	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	0	0	0	0	2	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	K	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	1	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	1	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	1	0	----	----	----	----	----	----	----	----	----	----	----

1911	Pair Number											
	1	2	3	4	5	6	7	8	9	10	11	12
September 20	0	1	0	0	1	1	0	0	1	0	1	------
21	0	0	0	0	D	0	0	0	0	0	0	------
22	0	0	0	0	------	0	0	0	0	0	0	------
23	0	0	0	0	------	0	0	0	0	0	0	------
24	0	0	0	0	------	0	0	0	0	0	0	------
25	0	0	0	0	------	0	0	0	0	0	0	------
26-27	0	1	1	0	------	1	0	0	0	0	0	------
28-29	0	0	0	0	------	1	0	0	0	0	0	------
Totals	121	447	314	53	328	413	111	185	256	78	409	320

Pair Number																						
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	1	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
0	0	----	----	0	0	----	0	0	0	0	0	----	----	----	----	----	----	----	----	----	----	----
65	212	292	30	73	120	166	27	296	343	196	39	13	23	10	21	9	10	12	24	4	8	8

TABLE VI.

SUMMARY OF EGG LAYING RECORDS, BY MONTHS FROM TABLE V.

Pair Number	May	June	July	August	September	Totals
1	11	76	28	6	0	121
2	15	102	146	132	52	447
3	5	83	129	83	14	314
4	10	37	6	0	0	53
5	10	144	106	57	11	328
6	11	126	139	108	29	413
7	14	63	28	6	0	111
8	13	71	95	6	0	185
9	4	44	99	86	23	256
10	7	71	0	0	0	78
11	7	122	131	119	30	409
12	13	140	158	9	0	320
13	6	34	4	21	0	65
14	8	60	109	35	0	212
15	9	148	116	19	0	292
16	5	25	0	0	0	30
17	15	50	8	0	0	73
18	8	90	22	0	0	120
19	12	35	80	39	0	166
20	5	20	2	0	0	27
21	13	102	83	90	8	296
22	7	79	128	108	21	343
23	10	53	41	67	25	196
24	4	12	16	7	0	39
25	7	6	0	0	0	13
26	11	12	0	0	0	23
27	3	1	6	0	0	10
28	6	6	9	0	0	21
29	5	4	0	0	0	9
30	6	4	0	0	0	10
31	0	8	4	0	0	12
32	9	15	0	0	0	24
33	4	0	0	0	0	4
34	6	2	0	0	0	8
35	0	4	4	0	0	8
Totals.....	279	1,849	1,697	998	213	5,036

TABLE VII.

PAIRS NUMBERS 1 TO 23. COLLECTED ON PENDER

Eggs Laid		1	2	3	4	5	6	7
June	16	3	3	2	4	0	0	3
	17	2	4	2	0	0	0	1
	18	2	2	2	2	0	0	5
	19	0	1	1	1	0	0	4
	20	1	0	2	2	0	0	6
	21	2	2	2	1	1	0	6
	22	1	2	1	3	0	1	3
	23	2	2	2	1	0	0	1
	24	0	1	0	1	0	1	4
	25	2	4	0	2	0	1	10
	26	1	3	0	2	0	0	3
	27	1	5	0	7	1	0	2
	28	0	2	0	1	0	0	1
	29	3	3	0	2	0	1	5
	30	2	0	0	2	1	1	5
July	1	0	1	0	1	0	0	3
	2	0	0	0	1	0	0	1
	3	0	1	0	0	0	0	3
	4	2	3	0	8	0	0	9
	5	1	3	0	3	1	5	1
	6	0	2	2	0	0	1	0
	7	2	4	0	5	0	4	1
	8	3	2	1	4	1	5	8
	9	3	3	1	0	0	1	3
	10	1	1	0	5	0	1	8
	11	1	2	2	2	1	0	4
	12	3	2	0	4	0	7	11
	13	0	1	2	0	0	2	4
	14	4	1	2	3	0	2	7
	15	3	1	2	9	0	7	3
	16	4	3	3	7	0	2	4
	17	2	2	1	9	0	1	4
	18	3	0	6	5	0	3	7
	19	3	4	9	4	1	1	4
	20	1	1	0	0	0	2	0
	21	5	2	5	1	0	2	4
	22	4	5	7	6	0	3	5
	23	5	3	3	7	0	5	5
	24	8	3	6	11	0	0	7
	25-26	2	4	6	5	1	3	4
	27	4	3	5	5	1	3	2
	28	4	3	8	6	0	0	1
	29	4	0	5	4	0	3	1
	30-31	7	2	3	8	0	1	4
August	1	3	1	4	3	0	1	5
	2-3	5	*	6	8	1	0	4
	4	5	2	5	4	1	3	4
	5	6	1	3	6	1	5	4
	6	2	2	4	3	0	3	1
	7	5	2	3	6	0	3	5
	8	4	2	4	7	1	2	4
	9-10	7	4	6	1	1	0	1
	11-12	3	3	4	5	0	2	6
	13	3	2	0	9	1	7	2
	14-15	8	1	0	2	2	1	2

---

*Ants.

Table VII.

TEST FARM, JUNE 13, 1914—ISOLATED JUNE 15, 1914.

8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
5	4	0	0	2	0	7	0	0	2	0	2	5	2	7	3
2	1	0	0	2	1	3	6	0	1	0	1	4	1	2	8
2	4	0	1	3	2	2	0	0	0	0	1	6	1	6	2
1	1	0	6	3	1	2	3	1	3	0	2	4	0	2	4
1	1	0	0	0	0	2	0	0	2	0	2	4	0	1	2
0	3	0	0	0	3	1	3	0	0	0	2	1	3	2	4
2	1	0	2	3	2	2	1	0	1	0	1	4	0	5	1
3	0	0	0	1	3	3	1	0	0	2	2	5	0	5	2
2	1	0	1	4	1	0	1	0	0	0	2	1	0	1	2
1	0	0	0	2	4	4	0	0	3	0	1	5	0	2	0
0	0	0	0	5	4	2	3	0	0	1	1	5	0	7	4
0	0	0	2	12	12	2	8	0	0	0	1	4	0	2	2
2	0	0	2	3	3	3	1	0	1	2	0	7	0	3	4
0	1	0	1	5	4	2	3	0	0	0	2	4	0	†	4
1	0	0	3	0	2	0	1	0	0	8	1	1	0	5	3
0	0	0	3	1	5	0	1	0	0	0	2	4	0	3	2
0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0
0	0	0	2	1	0	0	0	0	0	0	0	0	0	3	0
1	0	0	6	4	3	1	4	0	0	0	0	5	0	7	3
1	0	0	7	2	0	0	1	0	0	3	1	3	0	1	2
0	1	0	1	2	1	0	0	0	0	0	2	0	1	2	2
0	0	0	3	4	3	0	3	0	0	0	0	3	1	3	4
1	2	4	7	5	3	0	1	0	0	0	1	4	0	4	2
2	1	3	4	5	4	1	3	0	2	4	1	5	3	6	4
5	0	2	4	3	1	1	1	0	0	0	0	3	1	6	4
2	0	2	2	2	3	2	2	0	0	4	3	8	3	3	3
3	1	4	3	4	5	1	6	0	0	2	2	3	4	5	2
3	0	4	2	2	1	2	2	0	1	1	2	1	0	2	2
2	0	6	1	6	2	2	4	0	0	†	†	†	5	5	6
2	0	7	6	7	3	1	1	1	0	0	2	4	5	6	1
3	3	4	4	3	6	1	1	0	0	0	0	0	2	6	4
1	8	3	2	3	4	0	0	0	1	0	0	0	1	2	0
3	5	8	1	4	4	3	1	0	0	0	0	4	3	5	2
2	6	5	6	3	1	2	1	0	2	0	1	4	4	4	1
0	1	3	0	4	1	0	0	0	0	0	0	0	0	0	0
2	4	2	2	2	2	3	0	0	0	0	0	0	2	5	5
2	7	6	0	2	2	4	0	0	1	0	0	1	5	1	4
4	4	6	5	9	5	4	0	0	1	0	0	2	2	3	1
4	9	5	5	1	1	3	0	0	1	0	4	1	2	0	5
1	0	7	5	4	6	3	0	0	3	1	1	5	0	4	4
1	7	4	2	3	3	0	0	0	1	0	2	0	1	1	------
0	4	2	0	5	3	7	0	0	5	0	1	1	1	2	2
0	3	5	3	0	4	3	0	1	5	0	3	1	2	4	3
1	12	9	2	2	0	13	0	1	8	0	0	8	3	0	2
0	3	3	5	8	3	4	0	1	3	0	3	1	2	5	4
0	9	7	1	2	3	1	0	3	4	0	0	3	2	2	1
0	9	7	2	3	0	4	0	1	0	0	3	4	3	3	7
0	3	4	1	3	2	3	0	1	0	0	2	0	4	2	3
0	5	5	3	5	3	2	0	2	0	0	3	0	2	4	3
0	4	5	3	4	5	5	0	2	6	0	0	6	4	2	3
3	4	4	6	5	3	2	0	1	1	0	1	3	0	4	2
1	2	10	3	1	5	9	0	1	3	1	0	9	7	5	3
1	4	11	2	9	2	5	0	2	5	0	0	5	7	4	2
5	7	7	9	4	3	2	0	1	5	0	0	5	6	7	2
0	0	4	7	0	4	1	0	1	0	0	0	2	3	2	2

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†Without food.

Eggs Laid	1	2	3	4	5	6	7
August 18	10	3	3	3	1	0	9
20	0	0	3	1	5	3	2
24	0	1	0	0	0	3	0
Totals	162	120	137	211	23	102	229

8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	2	4	2	3	10	2	0	1	0	0	0	1	2	0	0
0	8	1	1	2	4	0	0	2	0	0	0	5	1	0	0
0	9	0	1	1	4	0	0	0	0	0	0	0	5	0	4
79	164	173	153	188	169	132	64	23	71	43	61	174	106	179	151

TABLE VIII.

SUMMARY OF EGG LAYING RECORDS, BY MONTHS FROM TABLE VII.

Pair Number	June	July	August	Totals
1	22	79	61	162
2	34	62	24	120
3	14	78	45	137
4	31	122	58	211
5	3	6	14	23
6	5	64	33	102
7	59	121	49	229
8	22	46	11	79
9	17	78	69	164
10	0	101	72	173
11	18	89	46	153
12	45	93	50	188
13	42	76	51	169
14	35	57	40	132
15	31	33	0	64
16	1	3	19	23
17	13	31	27	71
18	13	15	15	43
19	21	28	12	61
20	60	70	44	174
21	7	51	48	106
22	50	89	40	179
23	45	70	36	151
Totals	588	1,462	864	2,914

WHEN THE EGGS ARE LAID

There are two main periods of oviposition during the day, just as there are two main periods of other activities. These two periods center around 8 o'clock in the morning and 6 o'clock in the evening. In a large field where the bill bugs are very prevalent adult females can be found laying eggs almost any hour of the day or night. But adults laying eggs are much more abundant at the periods mentioned and are comparatively rare or sometimes seemingly wanting from about 12 o'clock noon until 3 in the afternoon and again from about 12 o'clock midnight to 5 in the morning. A fuller discussion of the daily activities of bill bugs is to be found under the heading "Diurnal Activities" below.

HATCHING

As the young larvæ develop within the egg their form gradually becomes more and more evident until it is fully outlined. Then the egg-shell seems to split just as if the developing larva was too large for it. The larva may actually eat its way out, but close examination of many egg-shells failed to give any evidence that this was the case. One example was watched while hatching and the splitting of the egg-shell seemed to be due to the active wriggling of the larva. If the egg-shell is actually eaten away by the larva, that fact could not be seen with the aid of a binocular microscope giving a magnification of 74 diameters.

Soon after hatching, the young larva commences to eat a tunnel downward and toward the center of the stalk.

PERCENTAGE OF EGGS HATCHING

During 1915, 37 different lots of eggs, making a total of 566 eggs, were watched in the insectary to determine the percentage of eggs that hatched. These eggs were placed in small tin boxes with sufficient green corn to give them plenty of moisture. Of the 566 eggs, 433 hatched, giving 76 per cent hatching. No reason can be assigned for the failure of the 24 per cent to hatch. All eggs that had not hatched by the end of nine days after laying were discarded and counted as not hatching, as they had usually molded by this time. No attempt has been made to compare this with conditions in the field, because no satisfactory method could be devised for watching the eggs under natural conditions.

Table IX.

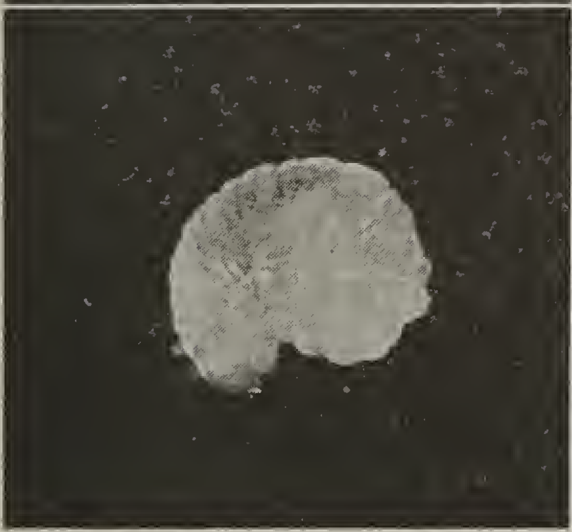
36 LOTS OF EGGS OBSERVED IN THE INSECTARY DURING 1915 TO DETERMINE PERCENTAGE HATCHING.

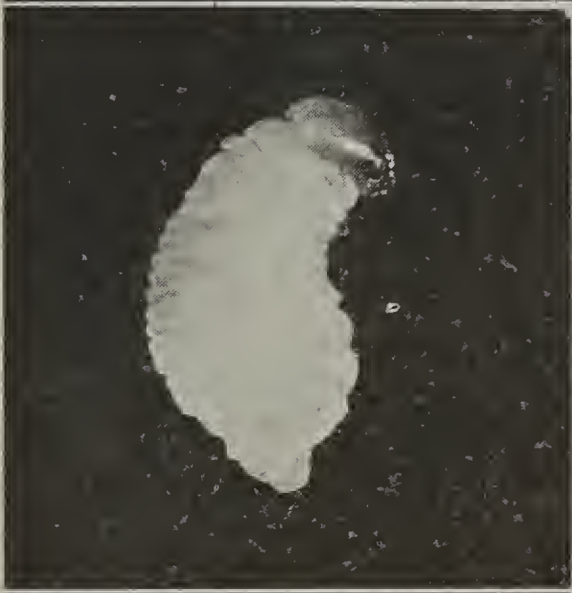
Lot Number	Number of Eggs	Hatched	Not Hatched	Date Laid
1	24	15	9	June 13
2	20	17	3	14
3	22	18	4	15
4	23	13	10	16
5	19	9	10	17
6	19	15	4	18
7	22	17	5	20
8	16	15	1	21
9	14	14	0	22
10	17	10	7	23
11	16	12	4	24
12	12	12	0	25
13	13	9	4	26
14	14	8	6	27
15	19	15	4	28
16	21	19	2	29
17	17	17	0	30
18	21	18	3	July 1
19	19	16	3	2
20	13	12	1	3
21	14	7	7	4
22	13	10	3	5
23	15	12	3	6
24	15	12	3	7
25	13	10	3	8
26	11	10	1	9
27	9	8	1	10
28	14	9	5	11
29	11	7	4	12
30	14	11	3	13

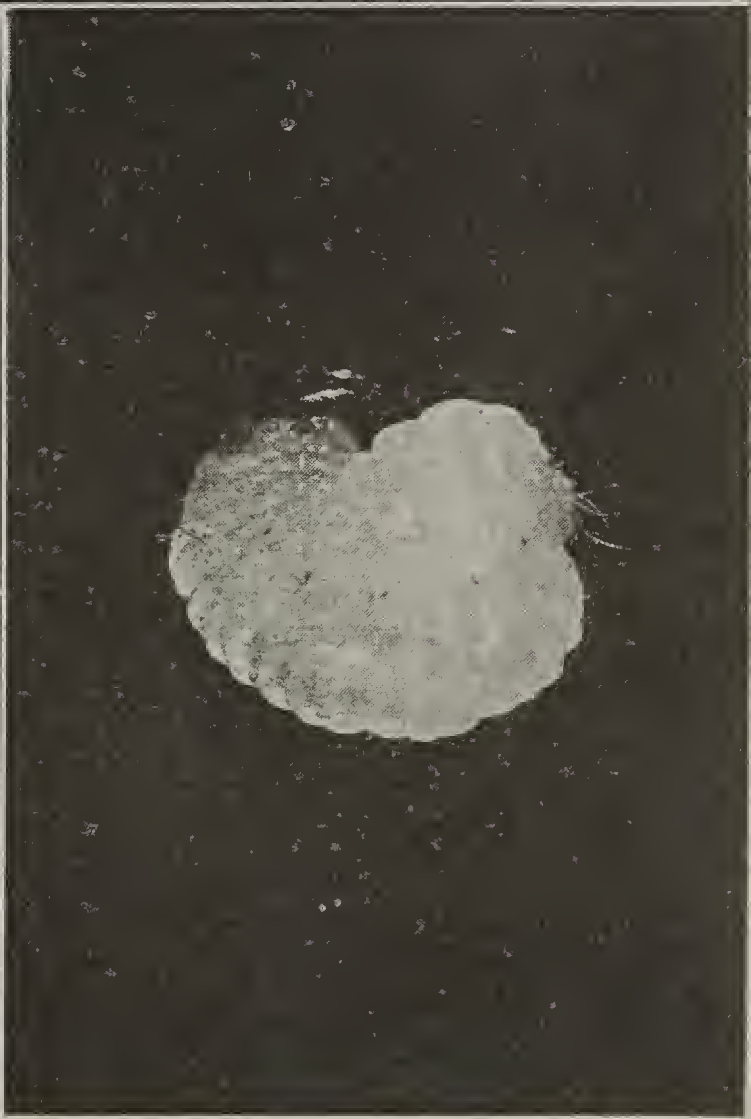
Lot Number	Number of Eggs	Hatched	Not Hatched	Date Laid
31	27	21	6	July 14-15
32	9	7	2	16-17
33	13	11	2	18
34	10	6	4	19-20
35	11	9	2	21
36	6	2	4	22
Totals.....	566	433	133	

THE LARVA DESCRIPTION

The larva of the Southern Corn Bill Bug is a fleshy footless grub with a yellowish brown head, much larger posteriorly (Figs. 19-22). The different molts are practically identical, there being no important differences save size. Length from 2 to 11 mm., depending upon the molt; soiled white; head yellowish brown; trophi darker, almost black; body fusiform, strongly curved, swelling ventrally from the third to the fifth abdominal segments, then recurved and rounding to the anal extremity. Head large, hemispherically oblong, longer than broad; epicranial suture distinct, continuing as a shallow impressed sulcus on the frons: a faintly indicated undulating line either side of the epicranial suture starting on the vertex and ending on the frontal sutures; frontal sutures distinct sinuate; epicranium with eight hairs either side of the epicranial suture arranged as shown in Fig. 23; frons with four hairs either side of median line as shown in Fig. 23; four shorter hairs on the clypeal margin; antennæ rudimentary; clypeus free, transverse trapezoidal, sides converging ventrad; labrum smaller, semicircular, bearing a row of four long medial, four short submarginal, and eight shorter marginal hairs on the ventral curved margin, two prominent curving sulci either side of middle, dividing the labrum into three sub-equal halves; mandibles stout, triangular, with two short hairs near the base; maxillæ stout with three prominent hairs, cardo small, stipes large, elongate, bearing a short palpus with two unequal globular joints and a fleshy lobe thickly studded with hairs, which is concealed by the labium; labium consisting of a large triangular mentum with a median excavation on the distal part, palpiger triangular with a deep median groove, labial palpi equaling the maxillary palpi of two joints, ligula triangular rounded; three pairs of prominent hairs on the mentum, one pair on the palpiger and two pairs of short stout hairs on the outer surface of the ligula. Thoracic segments distinct; prothorax longer and broader with a chitenized shield on the dorsum, a large oblong spiracle middle of the sides of the prothorax. A pair of rather prominent hairs dorsally with two smaller ones between, nearer the median line and in front of these two pairs near the anterior border.







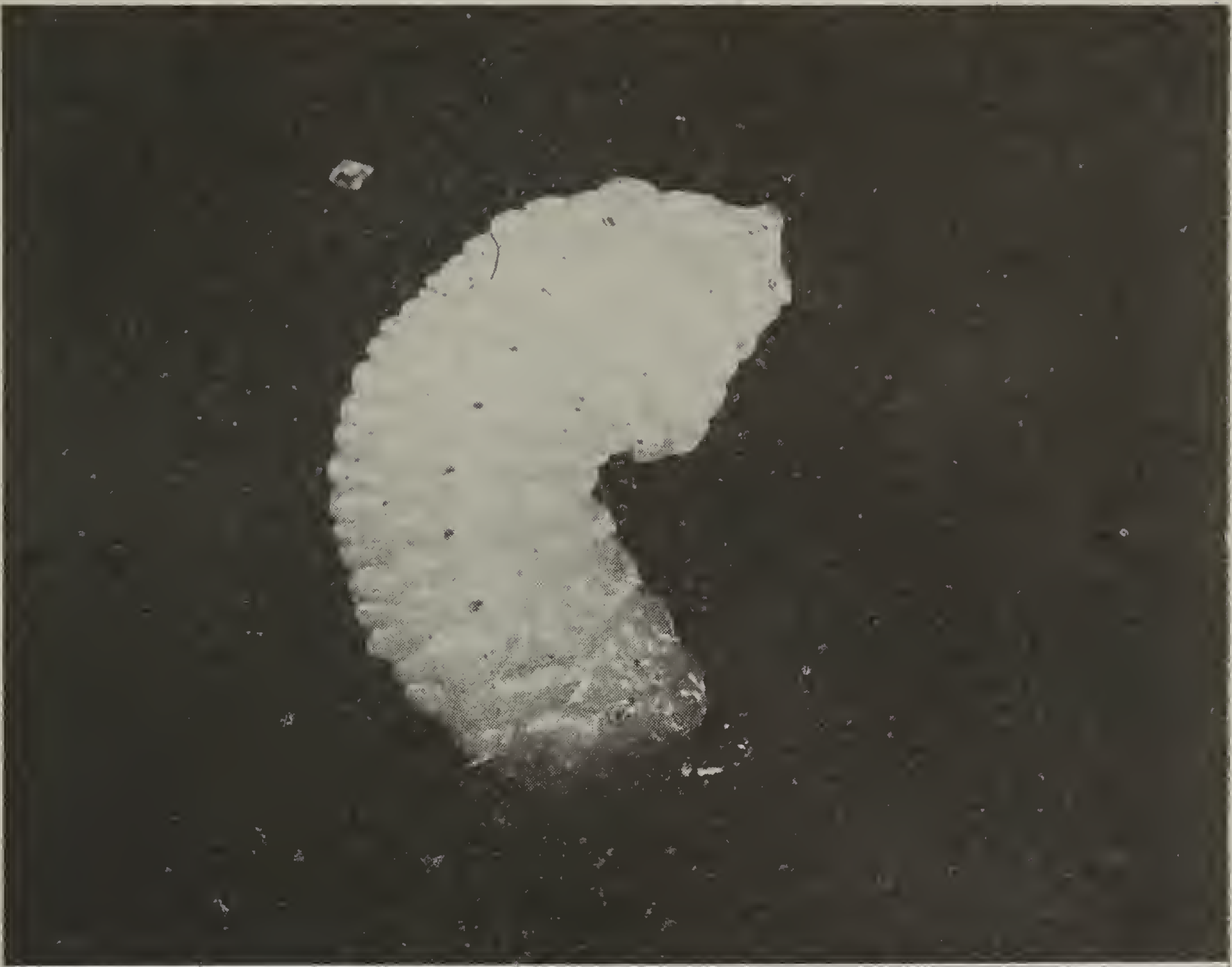


FIGURE 19. Larva just after first molt. X6.
FIGURE 20. Larva just after second molt. X6.

FIGURE 21. Larva just after third molt. X6.
FIGURE 22. Larva just after fourth molt. X6.

Posteriorly to the prominent pair there is a group of three hairs above the spiracle and four on the fleshy ventral protuberance. There is a pair each of the dorsal lobes of the mesothorax and metathorax. Three hairs on the dorso-lateral lobe of the mesothorax and two hairs on the metathorax. A single hair on each of the lateral lobes, ventro-lateral lobes, and the fleshy ventral protuberance of both the meso- and metathorax. The ventral lobes of each thoracic segment are provided with a median pair of hairs.

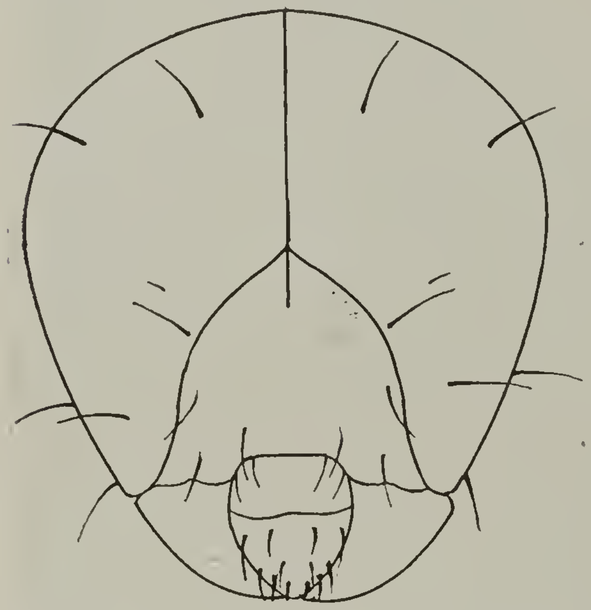


Figure 23. Frontal view of head of larva to show arrangement of hairs.

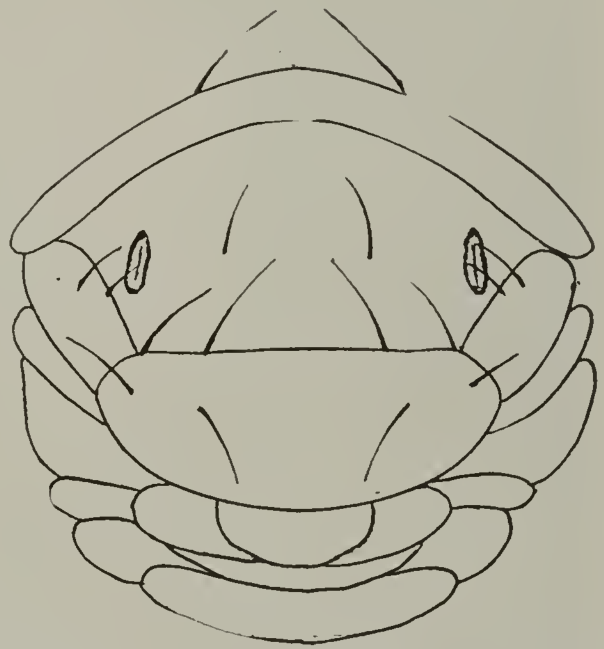


Figure 24. Seventh, eighth, and ninth abdominal segments of larva to show arrangement of hairs.

Abdominal segments forming on the dorsum a narrow transverse fold, separated by two wider folds; the eighth and ninth abdominal segments excavated above without dorsal folds; ventrally the first three segments contracted, the succeeding segments enlarged; laterally each segment has numerous longitudinal folds; abdominal spiracles smaller than prothoracic pair, eight pairs; the first seven pairs situated just above the lateral folds, the eighth pair situated in the excavation of the eighth abdominal segment; the eighth pair larger than the other abdominal spiracles, equaling the prothoracic pair; all with chitinous margins of dark brown color.

Each abdominal segment from one to seven has a pair of dorsal hairs, a single hair on each lateral and ventro-lateral lobe.

The arrangement of the hairs on the seventh, eighth, and ninth abdominal segments is quite characteristic, and is shown in Fig. 24.

METHODS OF REARING LARVÆ

Several different methods have been used for rearing larvæ in the course of this investigation. Practically all of our larvæ have been reared on the portion of the main stem of the corn plant just above the roots. In placing them in these pieces of corn stem, they were at first placed in a small cavity hollowed out of one side. Later it was found that it was much easier to watch them from day to day if the piece of cornstalk was split lengthwise, a small hollow large enough to receive the larvæ made in one piece and the two bound together with rubber bands. In this way the larvæ could be observed as often as necessary without undue disturbance, as the larvæ could usually be found in the central cavity which had been prepared for them.

Part of the larvæ have been reared in an out-of-door insectary (Fig. 25), and part have been reared in the laboratory (Fig. 28).

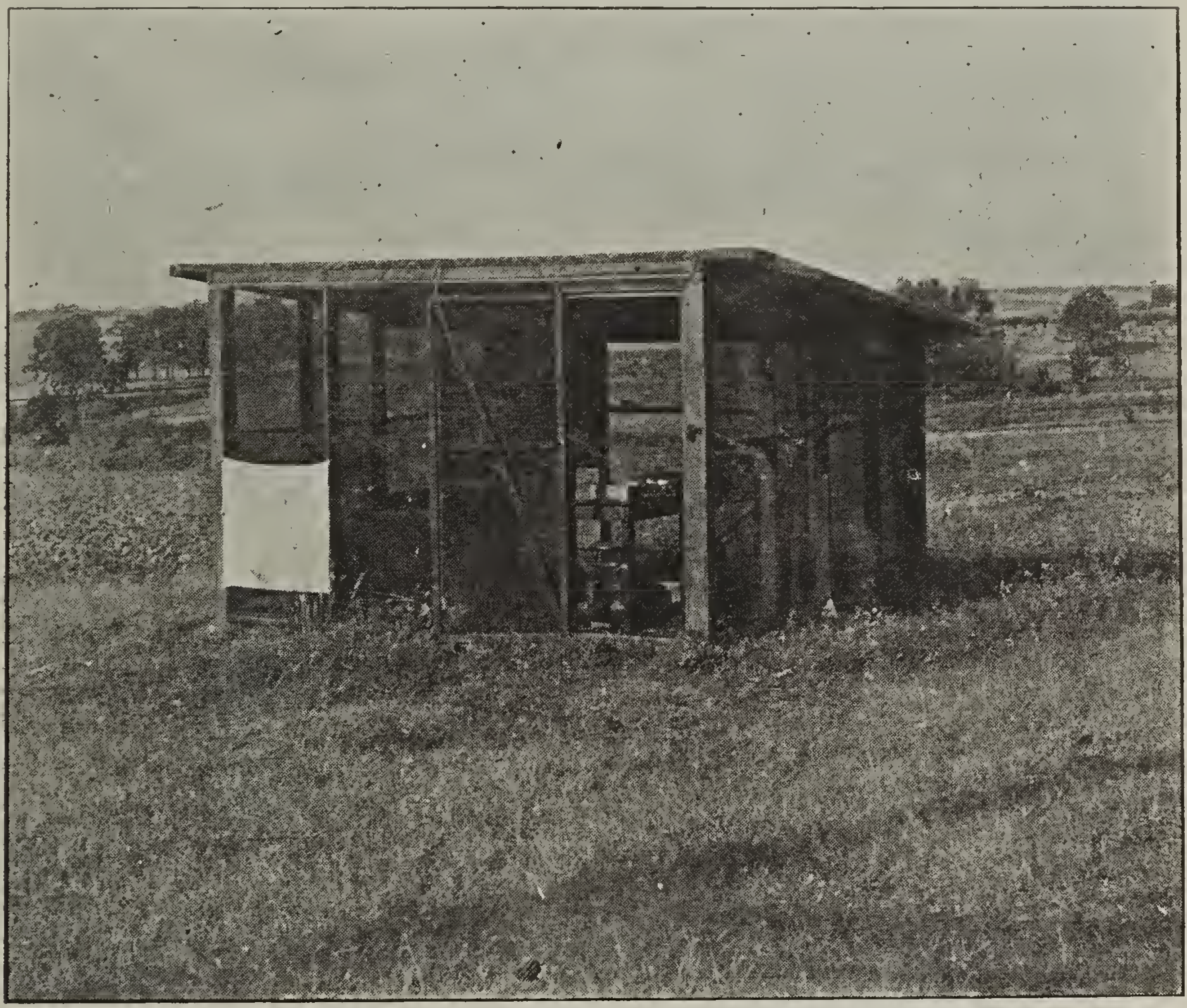


FIGURE 25. Insectary in which a part of the life history work on the Southern Corn Bill Bug was done. (After Smith.)

We have also reared larvæ in jelly glasses and in tin boxes, but of these two methods the tin boxes gave uniformly the better results and were much easier to handle. For this purpose we have used lately the Meyer's patent box, four-ounce size, which is provided with a permanent label on the lid (Fig. 27). This label was very convenient for making any records regarding the larvæ contained in the box, such as time and date of last observation, when last provided with food, date and time of molting, etc. Such records as were worthy of being permanently filed were put on sheets and filed in a vertical file. In this way there was no danger of confusing the record of one larva with another, and the complete record was always before the observer while examining any individual.

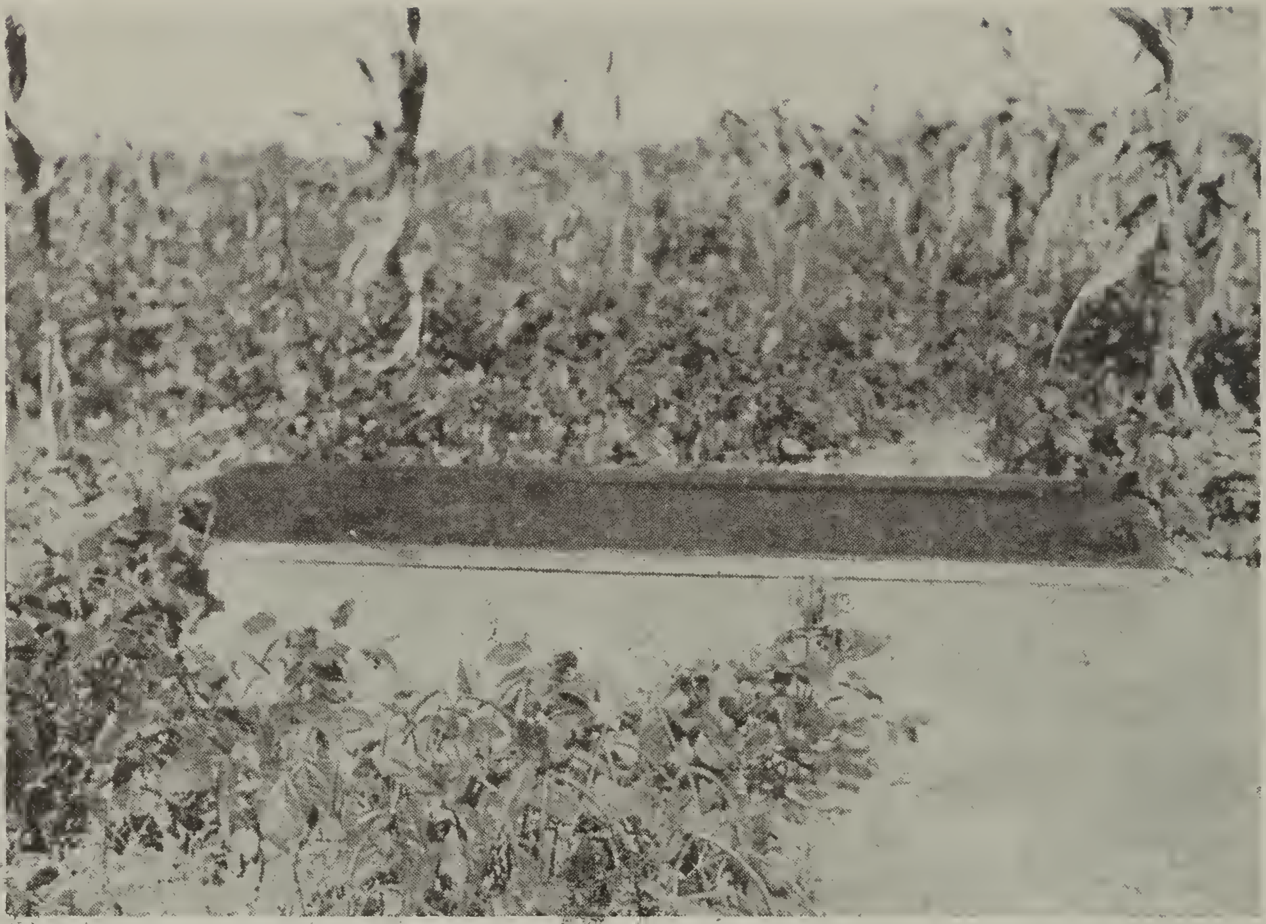


FIGURE 26. Hibernation cages used in the fields to determine the length of the hibernation period.

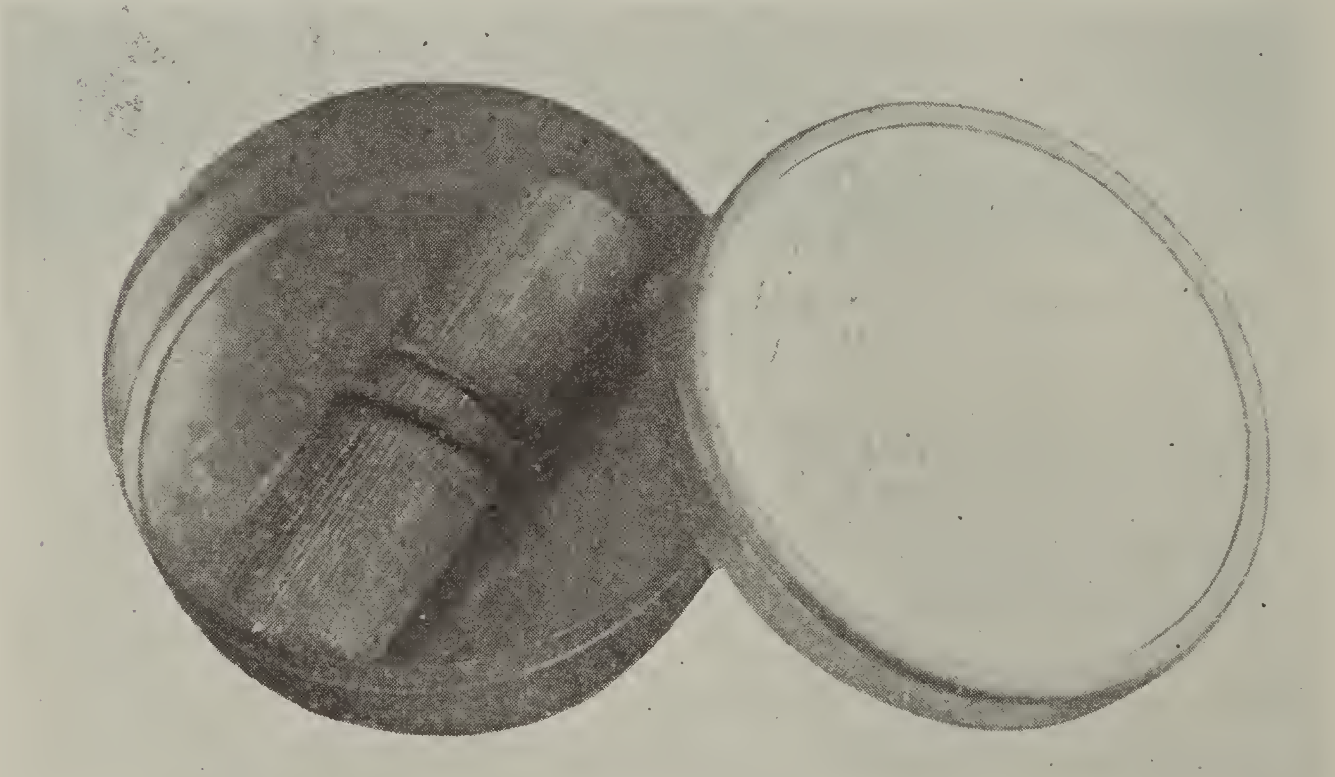


FIGURE 27. Tin salve box used in breeding the Southern Corn Bill Bug in the laboratory.

In the jelly glasses and in the tin boxes the pieces of cornstalk containing the larvæ were placed on and buried in damp soil, and placed on and buried in dry soil. Towards the end of the investigation all of these methods were discarded and the larvæ were kept in boxes without any soil. In this way there was no danger of losing the larva if it happened to abandon its piece of cornstalk.

By this method 192 larvæ have been reared from egg to adult and many other larvæ have been reared through part of their development.

Very careful records have been made of these larvæ; in many cases the exact hour when they hatched, or when they molted, has been recorded. For many cases, however, this has not been recorded; therefore, in the following records the time has been recorded only to the day. In most of this work two observations were made daily, one from about 8 to 10 a. m., the other from about 5 to 7 p. m. In this way the day was roughly divided into two periods approximating twelve hours each. Most of the changes, however, occurred at night and are recorded for the morning following the night on which they were made, and in a majority of cases, therefore, accurate to within twelve hours.

PROCESS OF MOLTING

The process of molting has been watched in several larvæ. Briefly, it may be described as follows: The larva first becomes somewhat stiff and loses its characteristic curved shape and becomes more or less straight (Fig 29). This usually precedes the actual molting an hour or two, but in some cases the larva commences to assume this attitude as much as eighteen hours before it actually molts. The older the larva the longer relatively this preliminary attitude seems to be. In the case of the last molt or change to the pupa it is frequently as long as two or three days (Fig. 30). While in this attitude the larvæ turns and twists about a great deal. This is especially marked as the mature larva prepares to pupate. In this way the inside of the pupal cell is smoothed and made compact, and this same result is noticed in the larval burrows, to a much less degree, however (Fig. 29).

The second step in molting is the splitting of the head. The old head gradually splits along the epicranial and frontal sutures, and gradually, as the larva turns and twists, the new head emerges and the old headpieces are worked posteriorly. The chiten covering the rest of the body is gradually worked backwards, and is left eventually in the burrow behind the larva. This chiten is very thin and evanescent and soon loses its identity, but the harder headpieces, particularly the mouth-parts, persist for a day or two. This whole process normally occupies about half an hour, but frequently the larva seems to become exhausted and to lie quietly for an hour or so before recommencing its interminable turning and twisting.

The head after molting is pure white, but in the course of an hour it commences to turn yellow and gradually it assumes the brownish color characteristic of the larvæ between molts. This color is assumed very gradually, however, and frequently by close observation it is possible to distinguish a larva that has molted within the last twelve hours.

NUMBER OF MOLTS

In all, 221 larvæ have been raised from egg to pupal condition and many others have been carried through part of their development. Practically all of these larvæ evidently molted four times, but of this number only 93 were observed to make all their molts. A few individuals, fourteen in all, molted five times and three of these molted six times. From these observations it seems safe to conclude that the normal number of larval molts is four.

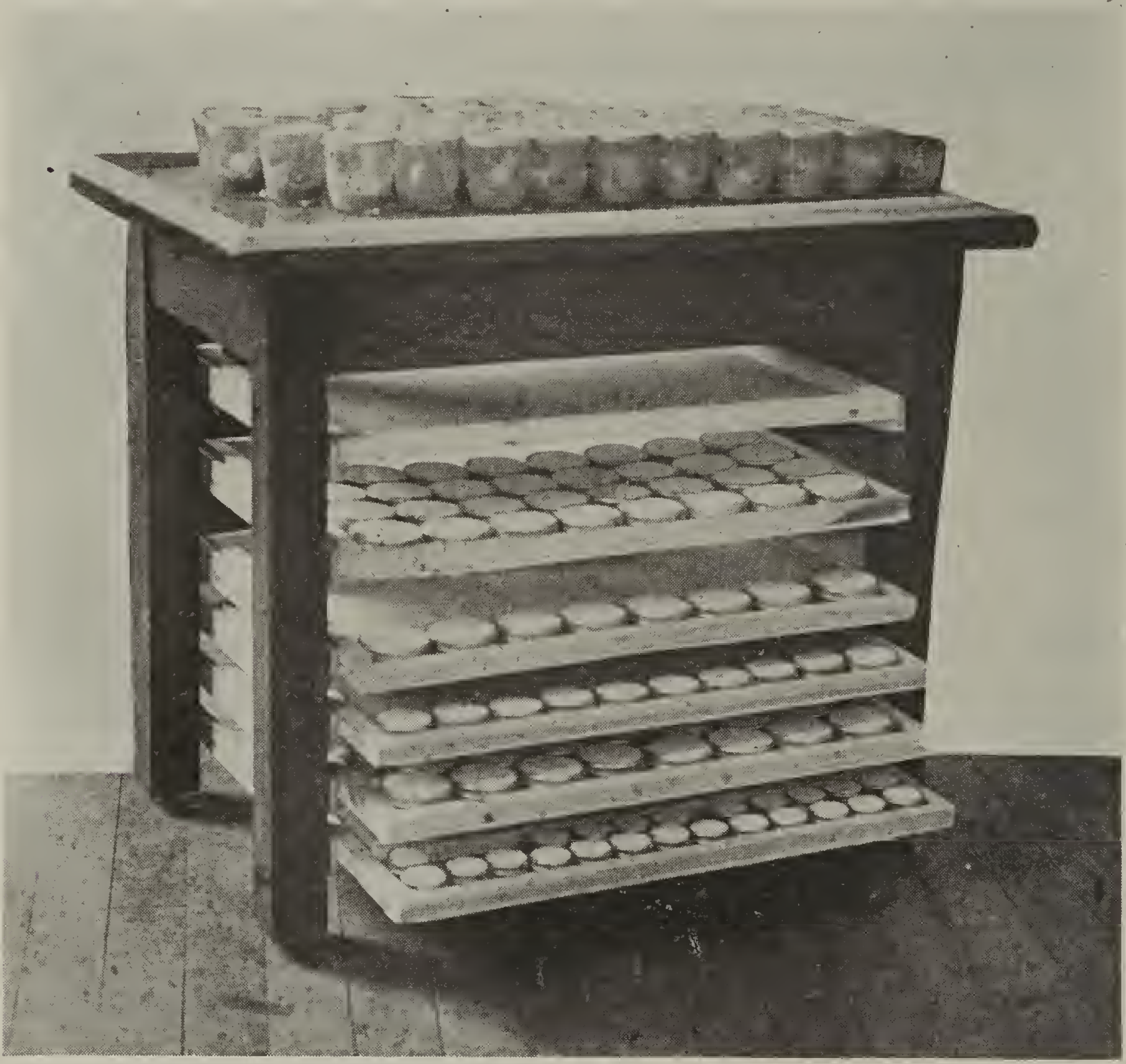


Figure 28. Laboratory table arranged with trays for holding jelly glasses and salve boxes used in breeding the Southern Corn Bill Bug.

Observations made on the above fourteen larvæ would lead one to believe that poor food would increase the number of larval molts. In one case the larva was purposely given poor food after its fourth molt. It molted six times. In another case a larva was purposely given poor food from the time it hatched, and it molted six times. Several

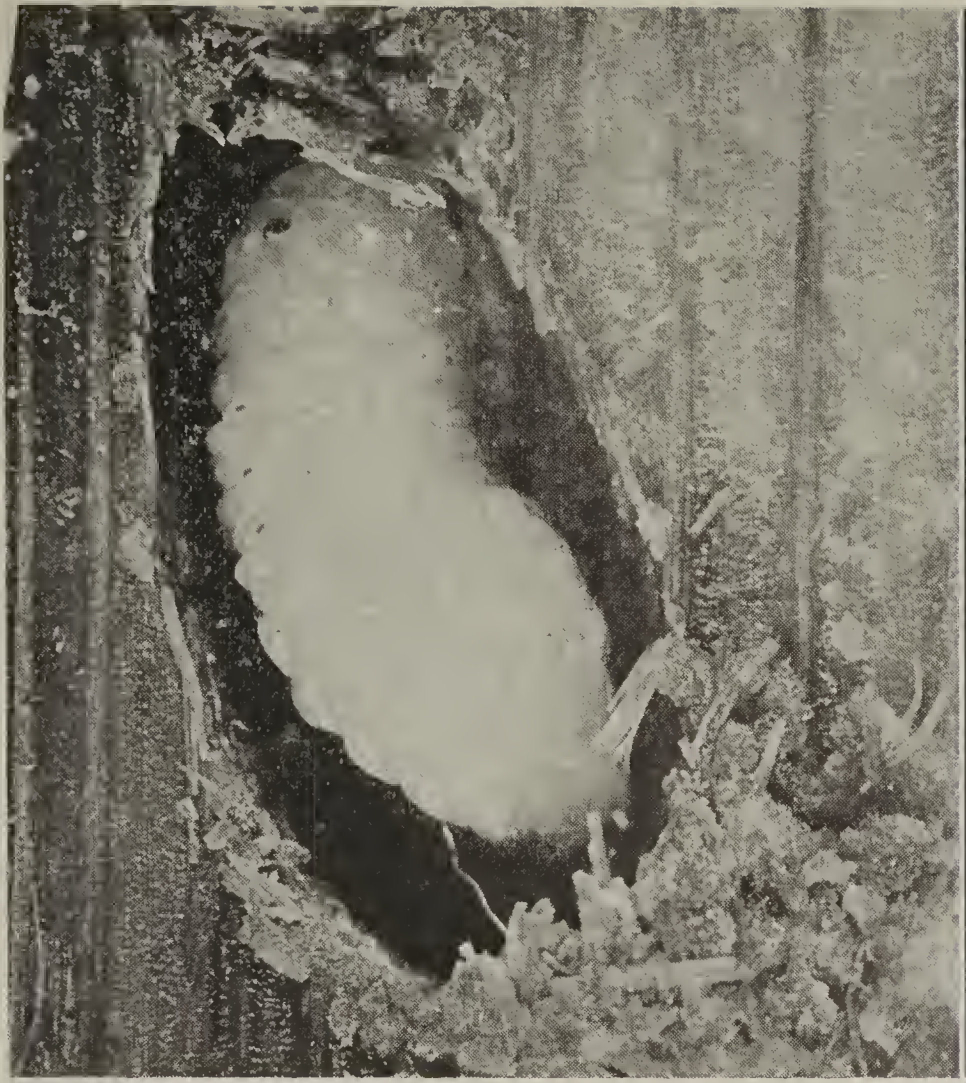


FIGURE 29. Larva preparing pupal cell. X6.

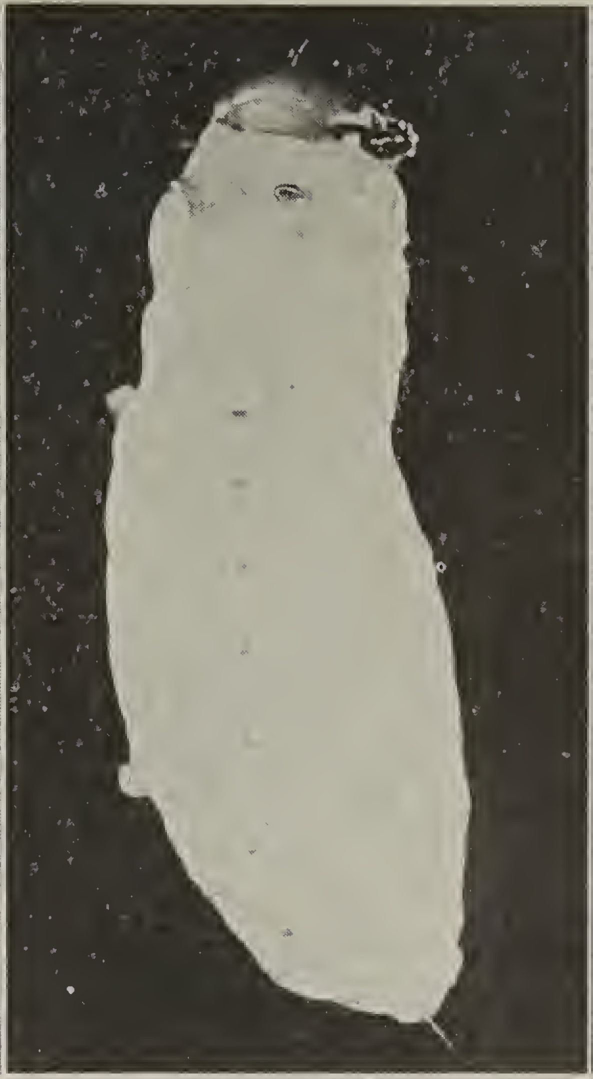


FIGURE 30. Larva just before fifth molt or pupation. X6

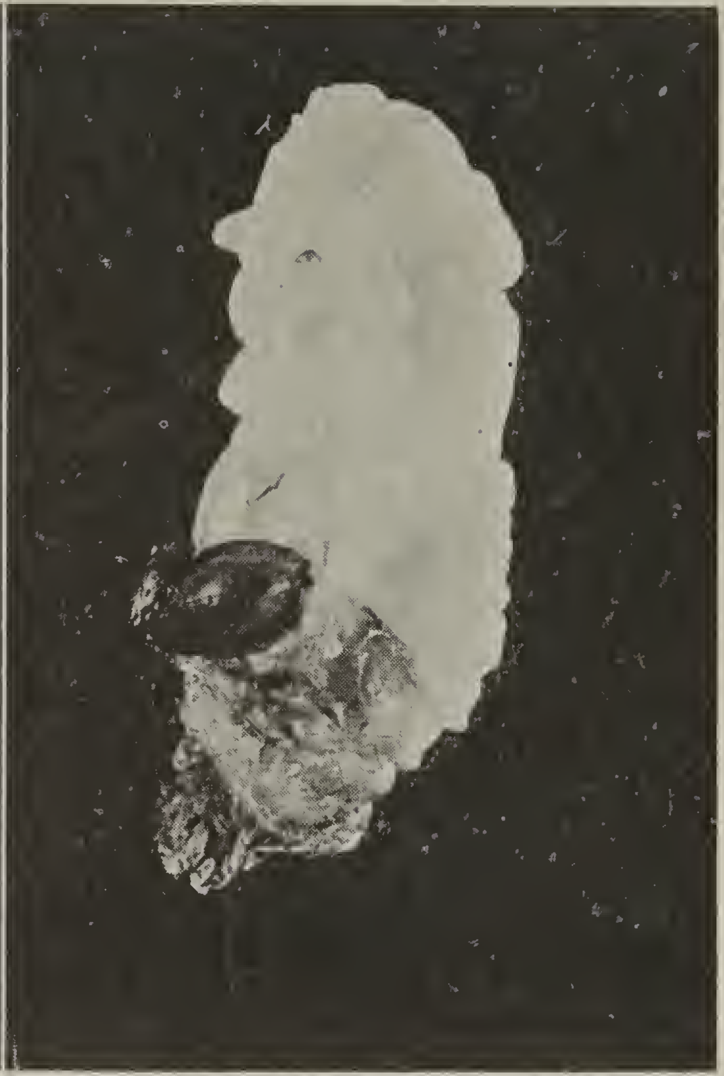


FIGURE 31. Pupa shedding larval skin. X6.

other individuals were tried in the same way, but we did not succeed in getting any more to pass through their stages, undoubtedly because the food was too poor.

In all our tables four has been taken as the normal number of larval molts, and all our calculations are based on these results. Whether the unnatural conditions of the insectary could have had any influence upon the number of molts is entirely beyond our power to determine. But, as will be pointed out in another connection, the writer believes that the conditions in the insectary as provided during these investigations was near enough natural conditions not to produce any unusual results. It is obviously impossible to follow the number of molts of an insect such as the southern corn bill bug under perfectly natural conditions, as normally it lives within its food plant.

Table X. LARVAL DEVELOPMENT.

Record Number	Date of Hatching	Date of Shedding				Date of Pupating	Number Days Larval Stage
		1st Molt	2d Molt	3d Molt	4th Molt		
1	June 3	?	June 24	?	July 13	July 22	48
2	June 4	?	?	?	July 1	July 8	34
3	June 4	?	?	?	?	July 8	34
4	June 8	June 13	June 26	July 6	July 11	July 19	40
5	June 9	?	?	?	July 4	July 14	35
6	June 8	June 13	June 20	?	July 1	July 11	32
7	June 9	?	?	?	?	July 9	30
8	June 9	June 16	?	?	July 1	July 7	28
9	June 9	June 15	June 20	June 24	July 1	July 6	27
10	June 10	June 15	?	July 11	July 16	July 24	44
11	June 9	June 15	?	June 24	July 3	July 13	34
12	June 11	June 15	June 24	Jun 28	July 6	July 16	35
13	June 10	June 16	June 20	?	July 1	July 10	30
14	June 11	?	?	?	July 3	July 10	29
15	June 11	?	June 22	June 28	July 6	July 19	38
16	June 13	June 20	June 28	July 3	July 11	July 19	36
17	June 13	?	?	?	?	July 20	37
18	June 13	?	June 24	June 29	July 8	July 17	34
19	June 15	June 20	June 26	July 3	July 6	July 15	30
20	June 15	June 22	June 26	?	July 6	July 12	27
21	June 16	June 22	June 26	July 3	?	July 12	26
22	June 16	June 20	June 24	June 28	July 11	July 18	32
23	June 16	?	June 27	July 1	July 13	July 26	40
24	June 22	June 26	June 30	July 5	July 11	July 18	26
25	June 22	?	July 5	?	July 26	Aug. 7	47
26	June 22	June 29	?	?	July 11	July 22	30
27	June 22	June 29	July 3	July 12	July 20	Aug. 3	42
28	June 22	June 26	June 29	?	?	July 29	37
29	June 22	June 29	July 5	July 11	July 16	July 25	33
30	June 22	June 29	July 3	July 7	July 16	July 21	29
31	June 22	June 28	July 3	July 7	July 17	July 25	33
32	June 22	June 27	July 1	July 6	?	July 22	30
33	June 23	June 28	July 3	July 6	July 18	July 29	36
34	June 23	June 27	June 30	?	July 12	July 19	26
35	June 23	June 28	?	July 11	July 17	July 24	31
36	June 24	June 29	July 3	July 7	July 15	Aug. 17	54
37	June 26	?	July 5	July 11	July 15	July 25	29

Record Number	Date of Hatching	Date of Shedding				Date of Pupating	Number Days Larval Stage
		1st Molt	2d Molt	3d Molt	4th Molt		
38	June 26	July 1	July 6	July 11	July 17	Aug. 1	36
39	June 27	July 3	July 6	July 11	July 18	July 26	29
40	June 27	July 3	July 6	July 11	July 17	July 25	28
41	June 27	?	?	?	?	Aug. 3	37
42	June 27	?	?	?	?	July 31	34
43	June 28	?	?	?	?	July 26	28
43	June 28	?	?	?	?	July 26	28
44	June 28	?	July 12	July 17	July 26	Aug. 10	43
45	June 28	?	?	?	?	July 24	26
46	June 28	?	?	?	?	July 26	28
47	June 28	?	?	?	July 22	Aug. 2	35
48	June 28	July 4	?	?	?	July 27	29
49	June 29	?	?	?	July 23	Aug. 5	37
50	July 4	?	?	?	?	July 29	30
51	July 2	?	July 12	?	?	July 31	28
52	July 2	?	July 12	July 17	July 20	Aug. 5	34
53	July 5	?	July 17	July 22	July 26	Aug. 10	36
54	July 5	?	?	?	July 26	Aug. 6	32
55	July 7	July 13	July 17	July 20	?	Aug. 2	26
56	July 8	?	July 17	July 23	July 29	Aug. 10	33
57	July 8	?	July 19	July 23	July 29	Aug. 10	33
58	July 8	?	?	?	?	Aug. 7	30
59	July 8	?	July 19	July 22	July 27	Aug. 5	28
60	July 8	?	July 17	July 21	July 29	Aug. 10	33
61	July 12	?	?	Aug. 1	Aug. 10	Aug. 18	37
62	Aug. 3	?	?	Aug. 28	Sept. 5	Sept. 18	46
63	Aug. 7	?	Aug. 19	Aug. 24	Sept. 4	Sept. 12	36
64	June 2	?	?	?	?	July 14	42
65	July 8	?	July 17	July 24	July 31	Aug. 16	39
66	July 8	?	?	?	?	Aug. 11	34
67	June 10	June 16	July 20	July 1	July 5	July 13	33
68	June 9	?	?	July 8	July 11	July 19	40
69	June 22	?	July 5	July 16	July 18	Aug. 3	42
70	June 22	June 26	June 30	?	?	July 27	35
71	June 22	?	?	?	?	Aug. 14	53
72	June 23	?	?	?	?	July 20	27
73	June 26	July 1	?	?	?	July 31	35
74	July 24	?	?	?	?	Aug. 23	30
75	July 24	?	?	?	?	Aug. 26	33
76	Aug. 3	?	?	?	?	Sept. 11	37
77	Aug. 3	?	?	?	?	Aug. 26	23
78	Aug. 3	?	?	?	?	Aug. 26	23
79	Aug. 11	?	?	?	?	Sept. 6	26
80	Aug. 11	?	Aug. 27	?	?	Sept. 4	24
81	Aug. 28	?	?	?	?	Sept. 27	30
82	May 25	May 30	June 1	June 8	June 13	July 10	46
83	May 26	June 3	June 8	June 13	June 20	July 5	40
84	June 1	June 9	June 13	June 17	June 23	July 7	37
85	June 1	June 9	?	June 16	June 21	July 1	30
86	June 5	June 11	June 17	June 22	June 28	July 13	38
87	June 5	June 11	June 14	June 18	July 24	July 13	38
88	June 7	June 12	June 15	?	June 1	July 11	34
89	June 8	June 16	June 20	June 26	July 1	July 14	36
90	June 8	June 12	June 15	June 20	June 25	July 19	41
91	June 9	?	June 16	June 20	June 25	July 9	30
92	June 10	June 14	?	June 20	June 27	July 13	33

Record Number	Date of Hatching	Date of Shedding				Date of Pupating	Number Days Larval Stage
		1st Molt	2d Molt	3d Molt	4th Molt		
93	June 11	June 14	June 18	June 23	June 29	July 13	32
94	June 11	June 14	June 17	June 21	June 27	June 18	37
95	June 11	June 15	June 18	June 22	June 28	July 14	33
96	June 12	June 15	June 18	June 24	June 27	July 13	31
97	June 12	June 15	June 19	June 23	June 29	July 19	37
98	June 13	June 17	June 22	June 25	July 2	July 14	31
99	June 14	June 18	June 20	June 25	?	July 6	32
100	June 14	June 17	June 20	June 25	?	July 8	24
101	June 15	June 19	June 22	June 27	July 5	July 18	33
102	June 15	June 18	June 22	June 3	July 3	July 18	33
103	June 15	June 18	June 22	June 25	July 1	July 14	29
104	June 15	June 22	June 27	July 3	July 8	July 19	34
105	June 15	June 18	June 22	June 25	July 4	July 17	32
106	June 15	June 19	June 22	June 26	July 2	July 15	30
107	June 15	June 19	June 22	June 27	July 3	July 15	30
108	June 15	June 19	June 22	June 26	July 1	July 11	26
109	June 16	June 20	June 22	June 27	July 5	July 13	27
110	June 16	June 20	June 24	June 30	?	July 19	33
111	June 16	June 22	June 24	June 28	July 4	July 24	38
112	June 17	June 22	June 24	June 29	July 4	July 18	31
113	June 17	June 20	June 23	June 29	July 5	July 22	35
114	June 17	June 20	June 24	June 28	July 4	July 25	38
115	June 17	June 22	June 25	June 29	July 4	June 17	30
116	June 18	June 20	June 24	June 29	July 3	July 17	29
117	June 18	June 22	June 24	June 29	July 4	July 20	32
118	June 18	June 22	June 27	July 2	July 8	Aug. 1	44
119	June 18	June 22	June 26	July 1	July 8	July 24	36
120	June 18	June 22	June 27	July 1	July 8	July 21	33
121	June 18	June 22	June 25	June 25	?	July 14	26
122	June 19	June 22	June 27	July 2	July 11	July 24	35
123	June 19	June 22	June 25	June 29	July 6	July 20	31
124	June 19	June 22	June 25	July 1	?	July 19	30
125	June 19	June 22	June 28	July 3	July 9	July 19	30
126	June 19	June 23	June 27	July 3	July 11	July 24	35
127	June 19	June 22	June 27	July 1	July 7	July 20	20
128	June 19	June 22	?	July 2	July 10	July 22	33
129	June 19	June 22	June 28	July 2	July 8	July 21	32
130	June 19	June 22	June 27	July 2	?	July 25	36
131	June 19	June 23	June	July 1	July 8	July 22	33
132	June 19	June 22	?	July 1	?	July 15	26
133	June 19	June 22	?	July 2	July 8	July 22	33
134	June 20	June 24	June 28	July 2	July 9	Aug. 6	47
135	June 20	June 25	June 28	July 3	July 11	July 27	37
136	June 20	June 23	June 28	July 2	July 8	July 21	31
137	July 20	?	June 29	July 2	July 11	July 25	35
138	June 21	?	June 28	July 2	July 8	July 22	31
139	June 22	June 25	July 1	July 6	July 15	July 20	28
140	June 22	?	June 30	July 5	July 15	Aug. 3	42
141	June 22	June 28	July 8	July 15	July 18	Aug. 8	47
142	June 22	June 27	July 2	July 7	?	July 24	32
143	June 23	June 28	July 4	July 7	July 12	July 30	37
144	June 23	June 28	July 2	July 18	July 25	Aug. 7	45
145	June 23	July 1	July 4	July 11	?	July 27	34
146	June 25	June 28	July 9	July 11	July 20	Aug. 7	43
147	June 26	?	July 4	July 11	July 16	July 31	35
148	June 26	July 3	July 9	July 20	July 24	Aug. 6	41

Record Number	Date of Hatching	Date of Shedding				Date of Pupating	Number Days Larval Stage
		1st Molt	2d Molt	3d Molt	4th Molt		
149	June 27	July 1	July 4	July 11	July 17	Aug. 2	26
150	June 27	July 1	July 3	July 7	July 16	July 30	33
151	June 27	July 1	July 4	July 8	July 24	Aug. 3	37
152	June 27	July 1	July 5	July 12	?	July 24	27
153	June 27	July 1	July 4	July 9	July 24	Aug. 3	37
154	June 27	July 1	?	July 8	July 14	July 22	25
155	June 27	?	July 4	July 8	?	July 24	27
156	June 28	July 2	July 5	July 12	?	Aug. 5	38
157	June 28	July 2	July 5	July 9	?	July 21	23
158	June 28	?	July 5	?	July 21	July 30	32
159	June 28	July 2	July 7	July 12	?	Aug. 1	34
160	June 29	July 2	July 12	July 21	July 26	Aug. 10	42
161	June 29	?	July 5	July 12	July 22	July 26	27
162	June 29	July 3	July 12	July 17	July 26	Aug. 3	35
163	June 30	July 3	July 7	July 17	July 26	Aug. 3	34
164	June 30	July 3	July 8	July 20	?	Aug. 6	37
165	June 30	July 3	July 15	July 20	July 26	Aug. 9	40
166	June 30	July 3	July 8	July 17	?	Aug. 7	38
167	June 30	July 3	July 8	July 20	?	Aug. 3	34
168	June 30	?	July 8	July 15	?	July 31	31
169	July 1	July 10	July 15	July 22	July 27	Aug. 5	35
170	July 1	July 7	July 11	July 20	?	Aug. 14	40
171	July 1	July 9	July 15	?	?	July 30	29
172	July 2	July 9	July 15	?	?	Aug. 4	33
173	July 2	July 9	July 15	July 20	July 27	Aug. 12	41
174	July 2	July 5	July 12	July 15	July 21	Aug. 2	31
175	July 5	?	July 14	July 20	?	Aug. 2	28
176	July 5	July 9	July 15	July 22	July 30	Aug. 4	30
177	July 5	July 12	July 17	July 22	?	Aug. 6	32
178	July 5	July 9	July 15	July 18	July 26	Aug. 4	30
179	July 5	July 8	July 17	July 21	?	Aug. 4	30
180	July 5	July 9	July 15	July 22	?	Aug. 5	31
181	July 5	July 9	July 15	?	?	July 31	26
182	July 6	July 11	July 20	?	?	July 30	24
183	July 6	?	July 17	July 24	?	Aug. 10	35
184	July 7	July 16	July 18	July 26	Aug. 3	Aug. 14	38
185	July 7	July 12	July 17	July 26	July 30	Aug. 2	26
186	July 7	July 12	July 17	July 26	July 30	Aug. 8	32
187	July 7	?	July 20	July 26	?	Aug. 6	30
188	July 8	July 20	July 26	Aug. 2	Aug. 5	Aug. 18	41
186	July 8	July 14	July 20	July 26	?	Aug. 7	30
190	July 8	July 14	July 17	July 21	July 27	Aug. 9	32
191	July 8	?	July 22	July 22	July 30	Aug. 8	31
192	July 8	?	July 18	?	?	Aug. 2	25
193	July 8	July 15	July 18	July 22	?	Aug. 7	30
194	July 11	July 17	July 20	July 26	?	Aug. 6	26
195	July 11	?	July 20	July 26	?	Aug. 9	29
196	July 11	July 17	July 21	?	?	July 10	30
197	July 16	July 20	July 27	?	Aug. 4	Aug. 12	27
198	July 16	July 22	July 26	July 30	Aug. 6	Aug. 19	34
199	July 17	July 20	?	July 30	July 7	July 14	28
200	July 17	?	July 26	?	?	Aug. 14	28
201	July 17	July 21	Aug. 2	Aug. 8	Aug. 14	Aug. 20	43
202	July 17	July 21	July 27	?	?	Aug. 11	25
203	July 18	?	July 27	Aug. 2	Aug. 6	Aug. 25	38
204	July 20	?	July 30	Aug. 3	Aug. 7	Aug. 16	27

Record Number	Date of Hatching	Date of Shedding				Date of Pupating	Number Days Larval Stage
		1st Molt	2d Molt	3d Molt	4th Molt		
205	July 20	July 28	July 31	?	Aug. 6	Aug. 24	35
206	July 20	July 24	July 31	Aug. 3	Aug. 11	Aug. 24	35
207	July 20	?	July 30	Aug. 4	?	Aug. 24	35
208	July 20	July 24	July 31	Aug. 2	Aug. 6	Aug. 24	35
209	July 22	?	July 31	Aug. 7	Aug. 14	Aug. 21	30
210	July 22	?	July 31	Aug. 3	Aug. 9	Aug. 20	29
211	July 22	July 26	?	Aug. 7	Aug. 11	Aug. 23	32
212	July 22	?	July 31	?	Aug. 9	Aug. 21	30
213	July 24	July 31	Aug. 4	?	?	Aug. 16	23
214	July 26	July 31	Aug. 4	Aug. 9	Aug. 14	Aug. 29	34
215	July 26	?	Aug. 2	Aug. 4	?	Aug. 26	31
216	July 26	July 31	Aug. 2	Aug. 6	?	Aug. 29	34
217	July 26	July 31	Aug. 2	Aug. 4	Aug. 7	Aug. 29	34
218	July 26	July 31	Aug. 2	Aug. 6	Aug. 11	Aug. 23	28
219	July 27	July 31	Aug. 2	Aug. 6	?	Aug. 18	22
220	July 27	?	?	Aug. 4	Aug. 9	Aug. 26	30
221	July 27	July 31	Aug. 4	Aug. 6	Aug. 12	Aug. 22	26

DURATION OF THE MOLTS

(*First Molt, Fig. 19*)

In all, 150 first molts have been recovered from the 221 larvæ that have been reared from the egg to the pupa. The duration from the date of hatching to the first molt ranged from two to twelve days, the extremes occurring once each. The greatest number, 105 out of the 150 or approximately 70 per cent, molted between the third and fifth day. This is expressed graphically by the curve (Fig. 32). From these figures it seems safe to conclude that usually the first molt occurs some time between the third and fifth day from the hatching of the egg, but it may be greatly prolonged, due, perhaps, in most cases to poor food, and first molts occurring six, seven, and eight days after hatching are not usual, and the first molt may occur as long as twelve days after hatching.

There is perhaps greater regularity in the occurrences of the first molt than there is in any of the succeeding molts, due, perhaps, to the fact that the larva has had less time than in its later molts to be influenced by its environment, especially by favorable or unfavorable food supply.

The other factor that cannot be neglected in this matter is the fact that any irregularities in the first or succeeding molts are of necessity cumulative, that is to say, the chances are that any larvæ that molt a few days early or a few days late in the first molt would also molt irregularly for the second and succeeding molts; hence these irregularities would cumulate, and while individually they might be insignificant, yet when added to each other they have a tendency to throw our results away from the mean.

DURATION OF MOLTS

(Second Molt, Fig. 20)

There are two standards by means of which we may measure the duration of the second and succeeding molts. The first is the time that has elapsed since the first molt, and the second is the time that has elapsed since the egg hatched. Both of these methods have been used and the results expressed graphically by the attached curves (Figs. 33 and 34).

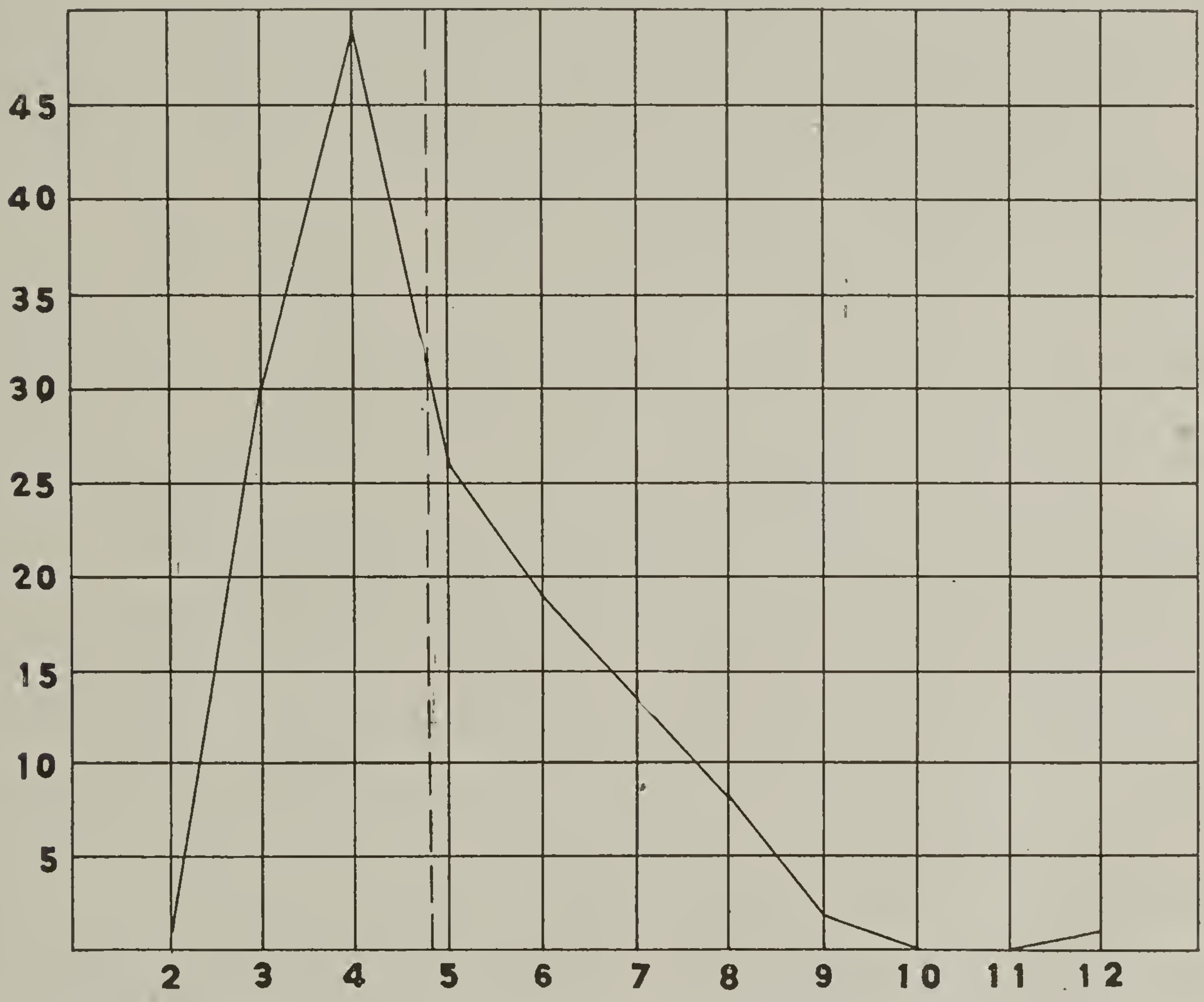


Figure 32. Curve showing the time lapsing between hatching and the first molt, one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

The time elapsing between the first and second molts is comparable to the time elapsing between hatching and the first molt, the range extending from 2 to 13 days. The majority, 106 out of 134 or a little more than 79 per cent, however, molted the second time from 3 to 6 days after the first molt.

The duration in days from the time of hatching to the second molt is not nearly as uniform as it is in the first molt, the range in 175 larvæ being 6 to 21 days. However, 140 larvæ out of the 175 observed molted a second time between the sixth and eleventh days, making a total of

80 per cent for six days, as contrasted with a total of 70 per cent in three days for the first molt. In the larvæ observed there was practically no difference in the numbers (thirty-one, twenty-four, twenty-seven, twenty-five, and twenty) molting on the seventh to eleventh days, inclusive.

DURATION OF MOLTS

(Third Molt, Fig. 21)

The range in time from hatching to the third molt for 165 larvæ out of 221 larvæ bred from egg to purpa has been found to be from 8 to 31 days. The majority, however, 103 out of 165 or 62 per cent, molted between the eleventh and fifteenth days, inclusive (Fig. 35), there being practically no difference in the numbers (eighteen, twenty-two, twenty-two, nineteen, twenty-two) molting on the eleventh and succeeding days to the fifteenth, inclusive.

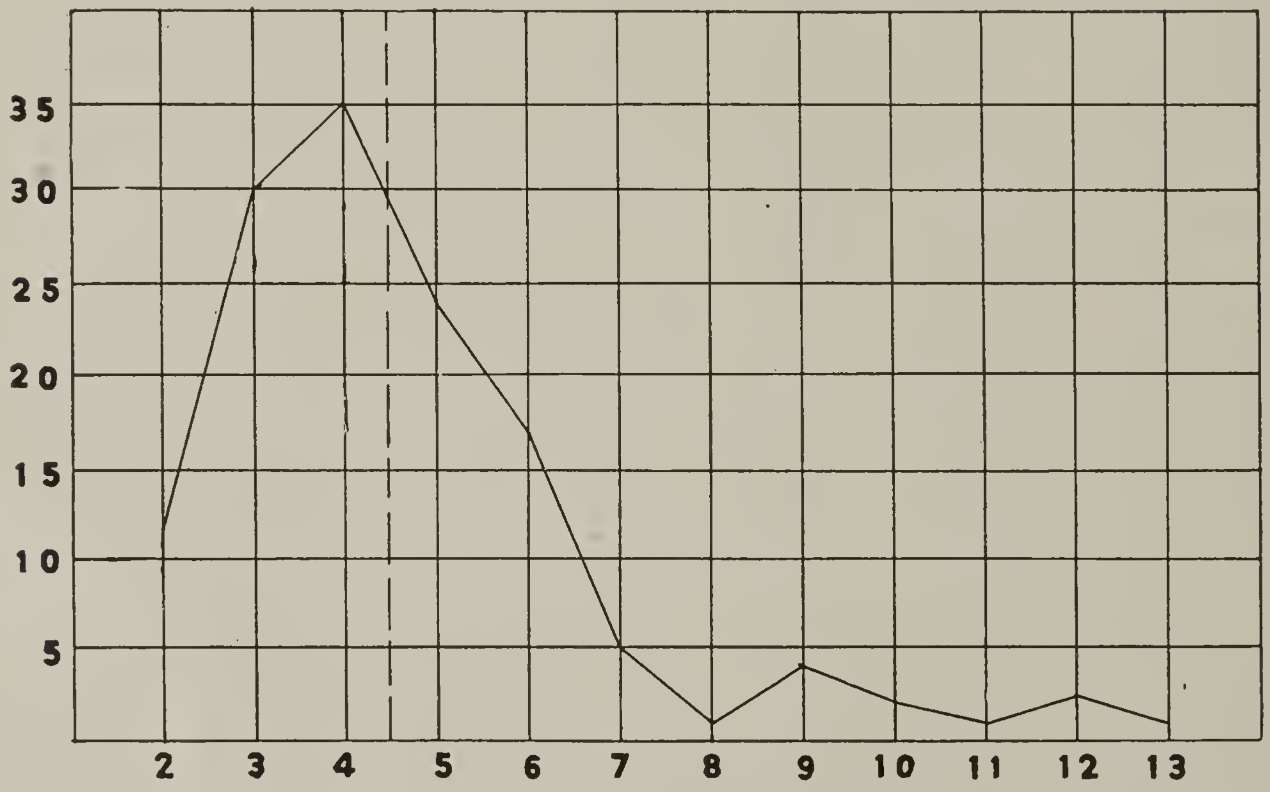


FIGURE 33. Curve showing the time lapsing between the first and second molts. one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

The time elapsing between the second and third molts is comparable to the time elapsing between hatching and the first molt and between the first and second molts, the range extending from two to sixteen days (Fig. 36). There was, however, only one larva out of 152 larvæ that required sixteen days between the second and third molts and none requiring thirteen, fourteen, or fifteen days. Hence this one larva may be discarded, giving us a range of from two to twelve days. These figures are very closely comparable to those found for the time elapsing between hatching and first molt and between first and second molt. In all, 115 out of the 152, or more than 75 per cent, molted for the third time between the third and sixth days after the second molt; the only striking point being the larger number, 83 or more than 54 per cent of the total number, molted between the fourth and fifth days after the second molt.

DURATION OF MOLTS

(*Fourth Molt, Fig. 22*)

The time elapsing between third and fourth molts ranges from 2 to 15 days (Fig. 37). Ninety out of the 130 larvæ, or about 70 per cent, molted between the fifth and eighth days, inclusive. Six days in this case is more pronounced than in any of the other curves for lapsed time (see Figs. 32, 33, and 36).

The total time from hatching to the fourth molt ranges from 12 to 40 days, and there is a great tendency to variation (Fig. 38). The principal mode seems to occur around the nineteenth day.

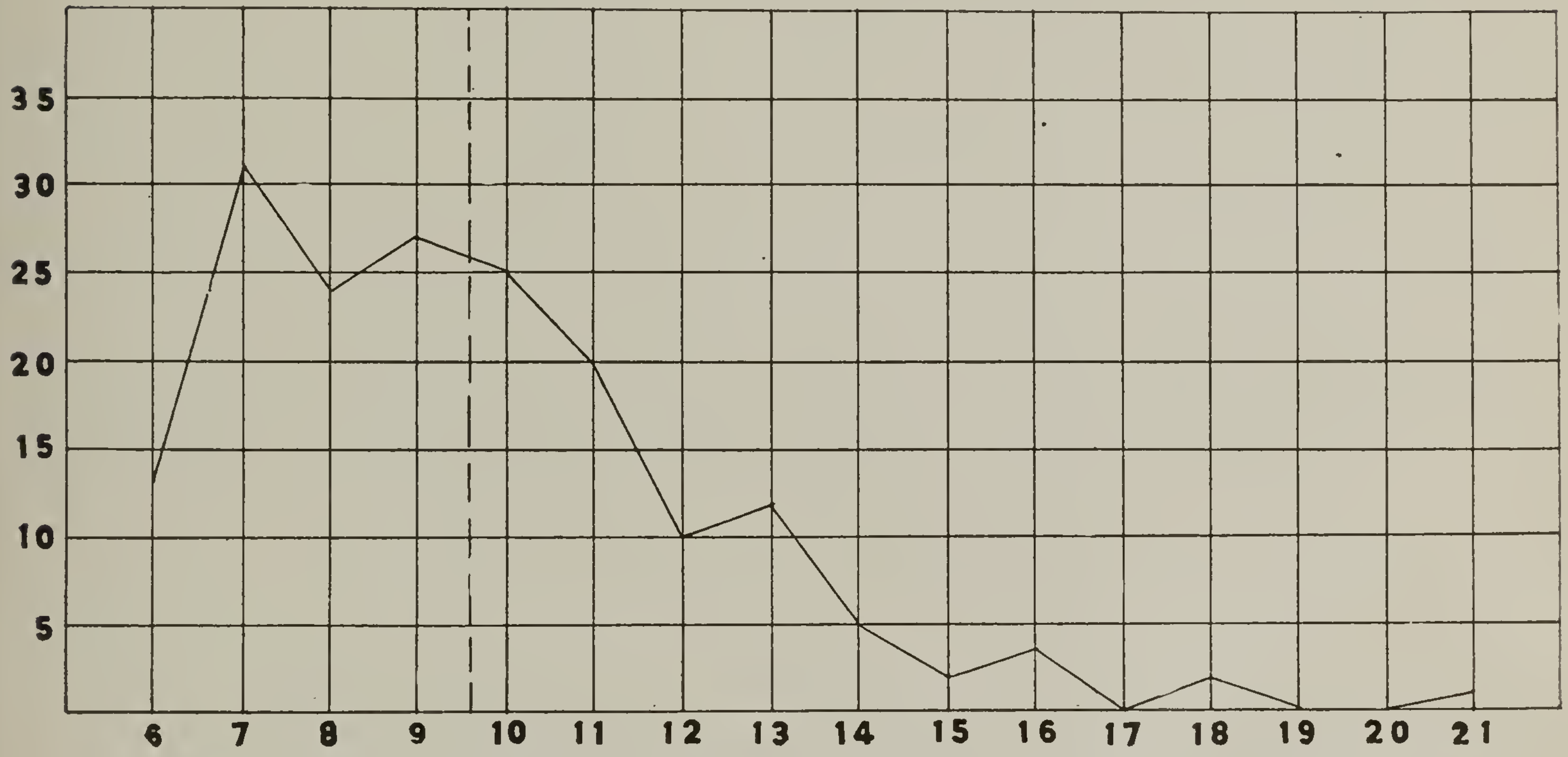


Figure 34. Curve showing the time lapsing between hatching and the second molt, one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

DURATION OF MOLTS

(*Fifth Molt, Fig. 30*)

(From fourth larval molt to pupation.)

The time between the fourth larval molt and pupation (fifth molt) is decidedly longer than that recorded for previous molts, ranging from three to thirty-one days. For the 149 larvæ for which we have been able to obtain the fifth-molt interval, there seems to be a tendency for the results to group themselves around two modes, one at 8 days and the other at 13 days. The mean, however, is 12.4 days, suggesting that the latter is perhaps the true mode, and that the apparent mode at 8 days is due to insufficient numbers (Fig. 39).

The total time for larval development, *i. e.*, from hatching to pupation, ranges from 22 to 54 days (Figs. 40 and 41). The majority,

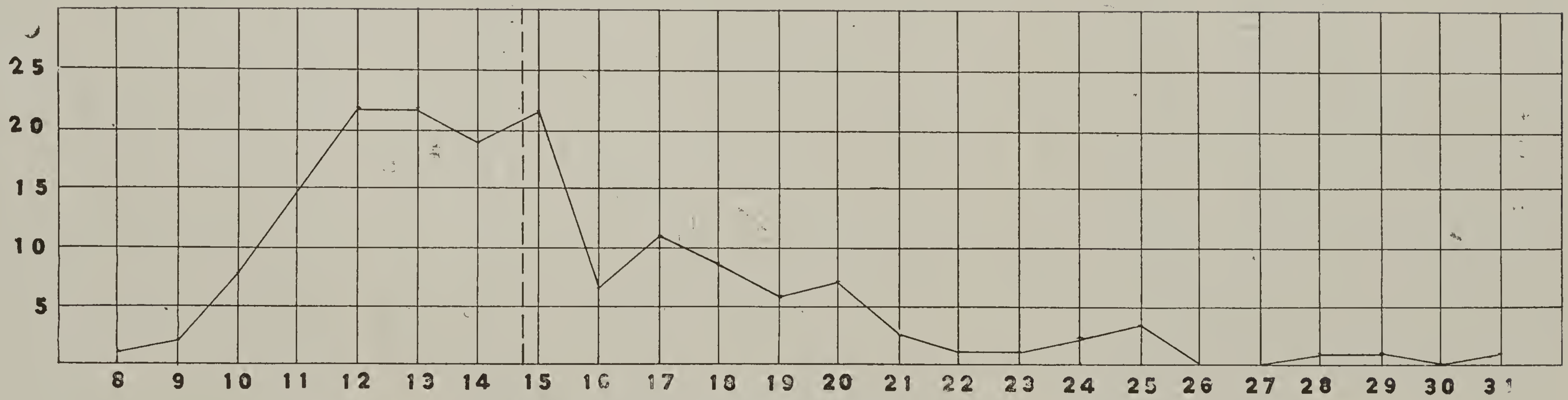


FIGURE 35. Curve showing the time lapsing between hatching and the third molt, one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

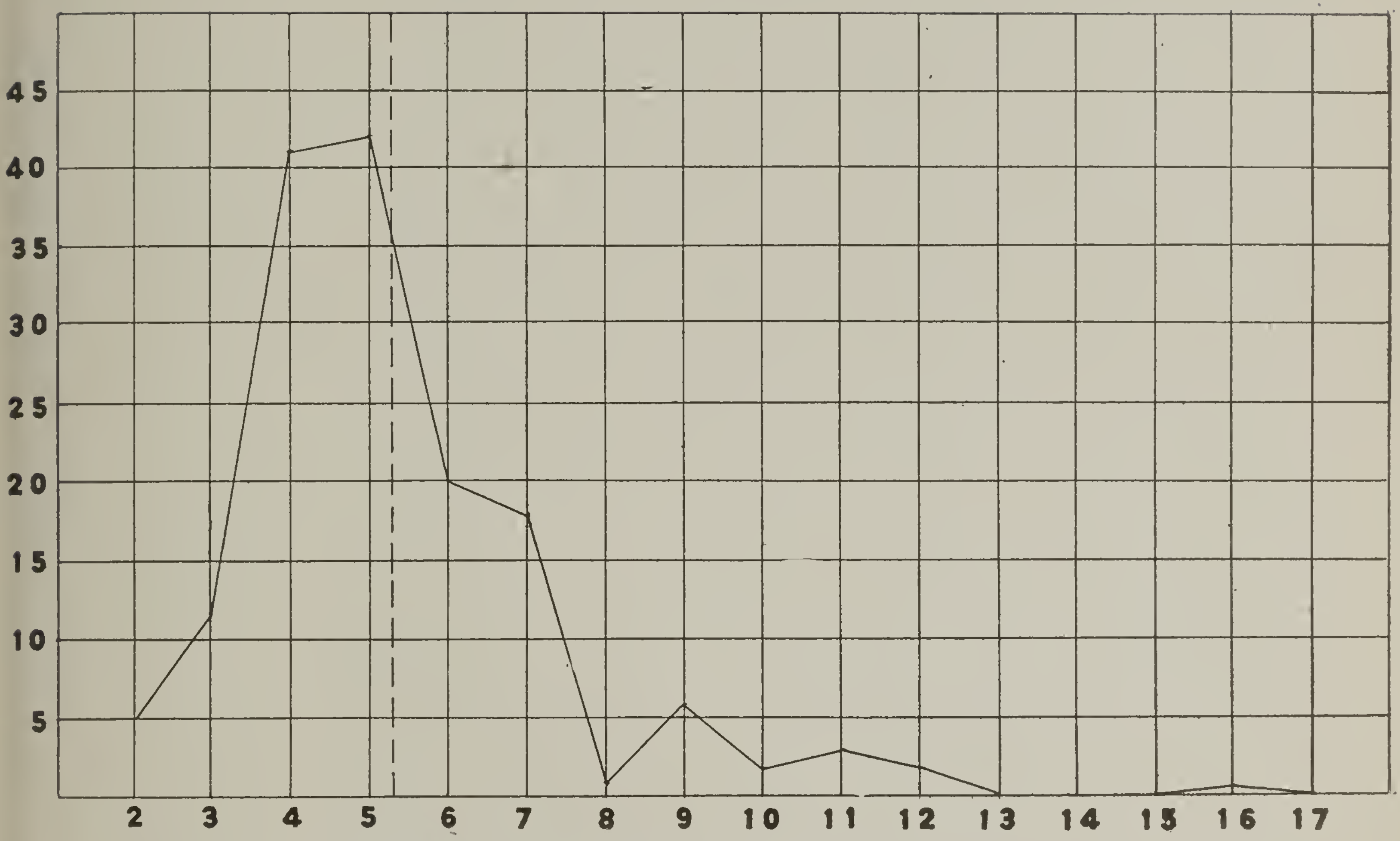


FIGURE 36. Curve showing the time lapsing between the second and third molts, one-day intervals. The mean time is shown by the dashed vertical line (— — — —).

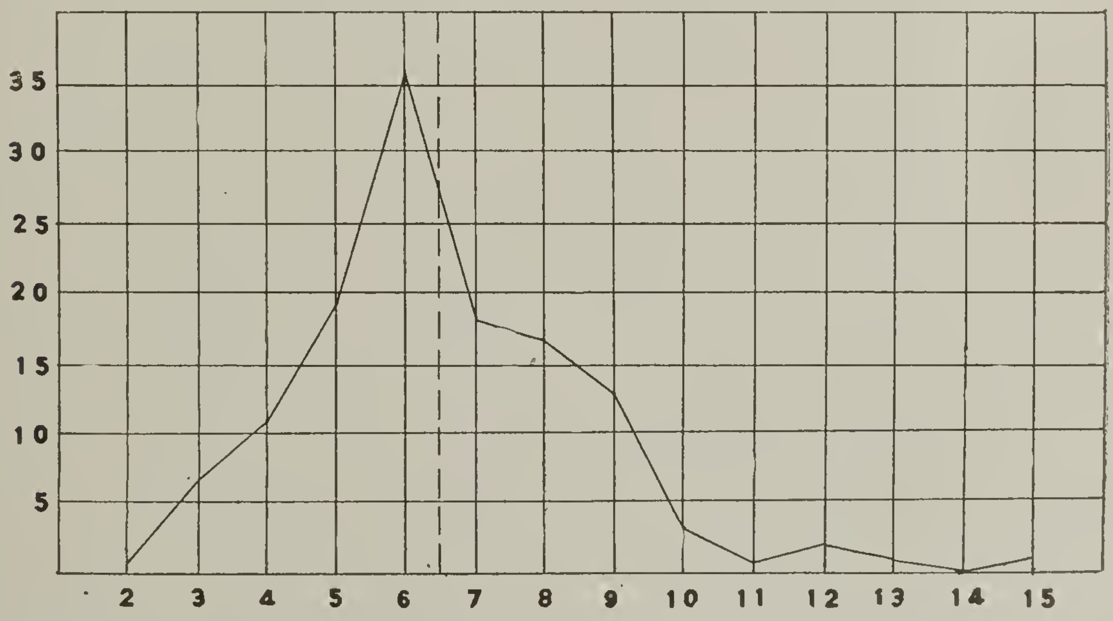


FIGURE 37. Curve showing the time lapsing between the time and fourth molts, one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

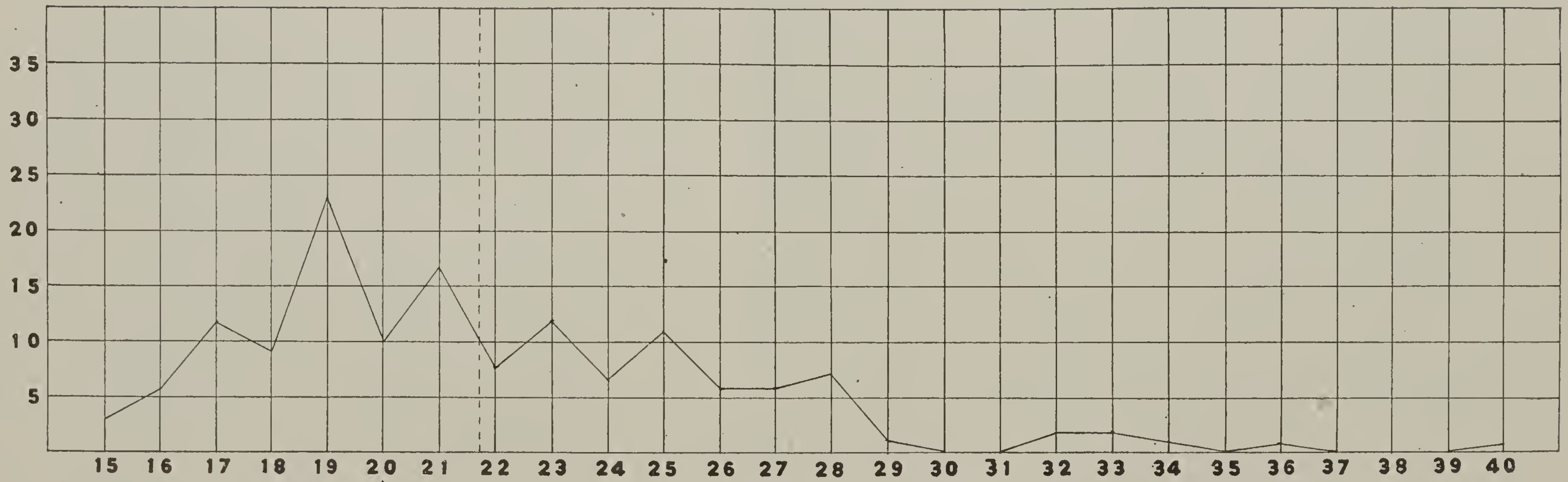


FIGURE 38. Curve showing the time lapsing between hatching and the fourth molt, one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

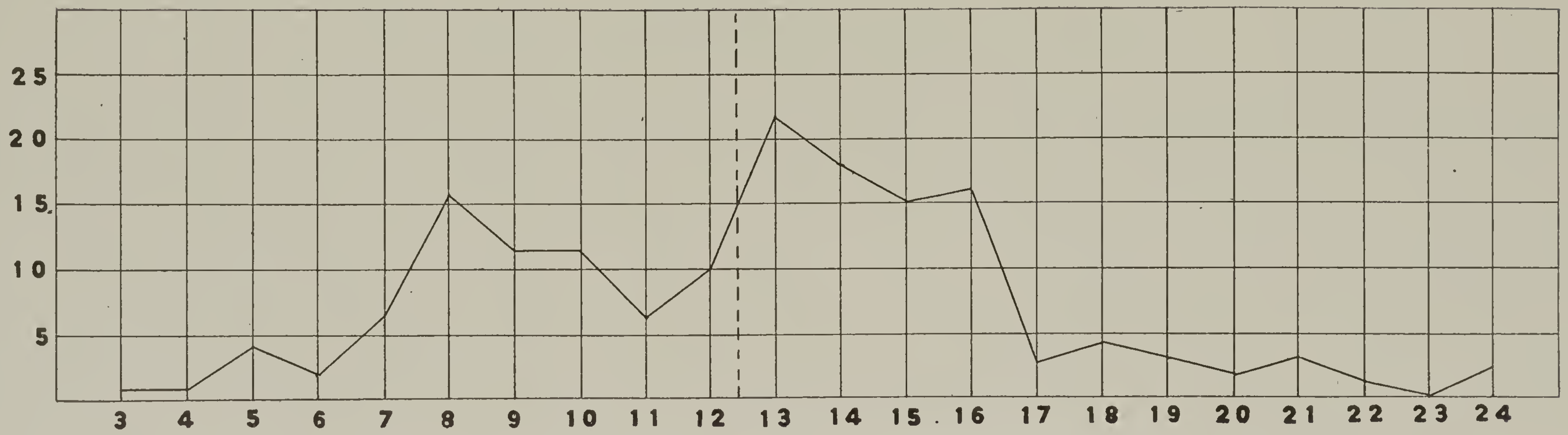


FIGURE 39. Curve showing the time lapsing between the fourth molt and pupation (fifth molt), one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

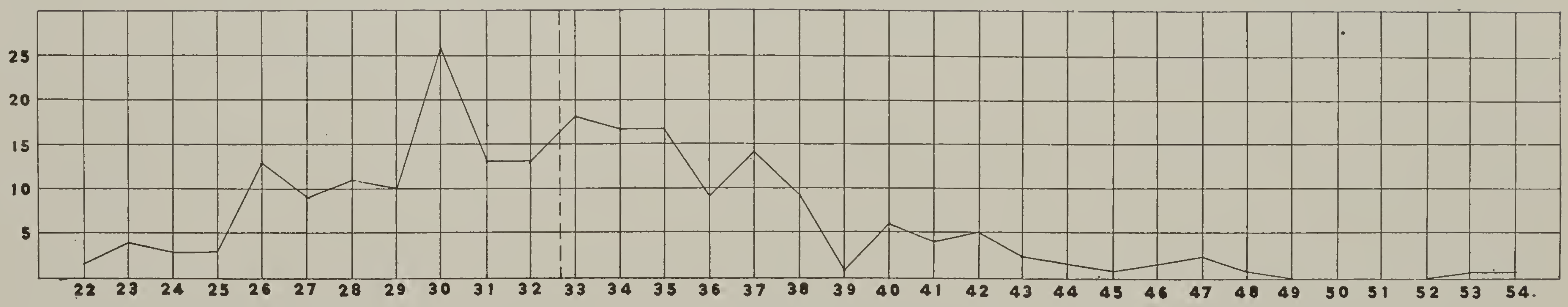


FIGURE 40. Curve showing the time lapsing between hatching and pupation (fifth molt), one-day intervals. The mean time is shown by the dashed vertical line (— — — — —).

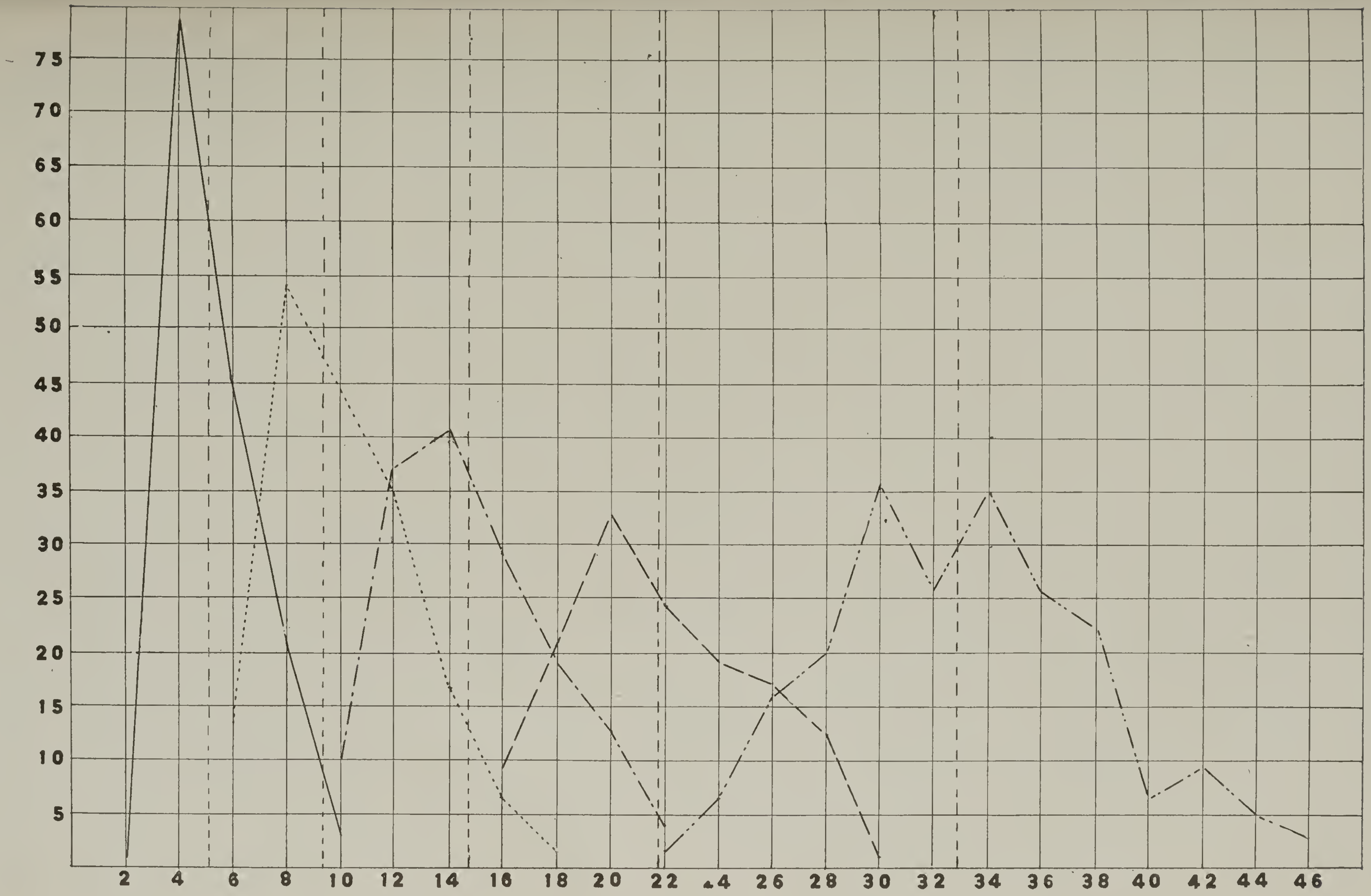


Figure 41. Curves showing larval development by molts, two-day intervals from hatching. Solid line first molt (————————). Dotted line (. . . . .) second molt. Dashed and dotted line (— . — . —) third molt. Dashed line (— — — — —) fourth molt. Dashed and dots (— .. — .. — .. —) fifth molt. The mean time for each molt is shown by the dashed vertical lines (— — — — —).

however, 169 out of the 221 larvæ observed, or about 77 per cent, passed through their larval development between the 26th and 37th days after hatching.

CHARACTER OF INJURY CAUSED BY LARVÆ

The most noticeable result of the presence of the larvæ in stalks of corn is the stunting of their growth. Nearly always in fields of any size there are some stalks that escape injury, due to a variety of conditions, and these stalks always tower above their neighbors like giants among a group of pigmies. Stalks that have worms present early in their development usually are injured to such an extent that they die. Stalks that are attacked later may grow to a certain extent after the attack by the larvæ, but one usually has no trouble in deciding which stalks in a given field are infested by larvæ and which ones are not.

These secondary effects upon the stalks are found in the abnormal growth that the stalks make. In addition to being stunted, the stalks, in severe attacks, are twisted out of all semblance to a cornstalk. This usually results in an attempt to produce suckers, but usually these suckers are not able to expand their leaves, and appear like some great abnormal growth on the sides of the stalk. (Figs. 42, 43, and 44.)

When the larvæ are working externally around the roots (Fig. 45), they seem to produce a distinct scar or split which extends up along the side of the plant, often to a height of several inches above ground. These scars are frequently shallow, but often they are very deep and seriously retard the normal growth of the plant. They appear to be out of all proportion both in length and in depth to the actual injury caused by the larvæ.

Another source of injury directly attributable to the larvæ is the weakening of the stalks to such an extent that they are blown over by heavy winds. This is more noticeable in fields that are only moderately infested by corn bill bugs than it is in fields that are badly infested, due to the fact that badly infested fields do not produce stalks large enough to be blown over by the wind.

Internally the larvæ upon hatching from eggs deposited in the stalk seem always to take a course diagonally inward and downward toward the center of the stalk, coming to lodge eventually in the tap-root (Fig. 46). While the larvæ are small, they do not seem to injure the stalk to any great extent, but as they increase in size they frequently eat away the entire tap-root (Fig. 47). This, of course, results in the stunting of the growth of the plant as noted above. This internal injury is nearly always confined to the tap-root and the portion of the stem below ground. Occasionally larvæ after working downward to the tap-root, turn and work upward sometimes to a considerable distance above ground. The causes behind such behavior are difficult to determine, as it certainly is not always due to a lack of food, for frequently larvæ will work upward in stalks where there is an abundance of food below ground, and again larvæ have been found in the tap-root where the entire root had been reduced to a dry reddish brown pulp. In some cases it appeared to be due to lack of moisture in the soil, as in all cases where the larvæ had been driven to feed above the ground the soil around the plant was very dry, whereas the stalk above ground was succulent and green. Whether this was the only factor is of course difficult to determine.

The writer believes that eggs laid loosely in the ground hatch into larvæ which feed externally on the roots and the stem below ground and do not enter the stalk. Some of these externally feeding larvæ may be from eggs that were laid in the stalk, the larvæ on hatching boring downward until they leave the tap-root, as it is not unusual to find stalks that larvæ have deserted in this way.

The larvæ, of course, always do much more damage to stalks of corn than the adults. This is exactly contrary to popular belief, due to the fact that the adults are more or less exposed and are more or less familiar to most farmers in the corn bill bug sections of the State, whereas the larvæ are hidden within the stalk and are not at all well known.

HABITS OF THE LARVÆ

Inasmuch as the larvæ work either in the stalk or among the roots in the ground, their habits are rather hard to follow consecutively.

The following notes are presented from a study of several hundred individuals collected in the field and from a more detailed study of the individuals reared in the laboratory.

The majority of larvæ seem to prefer to feed internally upon the tender growth just above the tap-root or in the tap-root itself. A considerable number of larvæ are to be found feeding externally upon the roots or the stem below ground. The writer believes that the larvæ that are feeding extenally are larvæ that have hatched from eggs which have been dropped loosely, a habit adult females seem to have after they have laid a number of eggs in the same day.

The larvæ that hatch from eggs deposited in cavities burrow downward toward the center of the stalk, enlarging their tunnels as they proceed. When the stalk is young and tender they seem to consume practically everything in their tunnels as they come to it, but in the older stalks the larvæ tear off large pieces, which are reduced to frass and left in the tunnel behind them. As the larva grows older it seems to tear off proportionally more and more of the stalk than it can devour, so that usually by the time it is ready to pupate the larval burrow is pretty well filled with fragments of stalk in the immediate vicinity of the larva, and it is from these fragments that the pupal cell is constructed.

The larva is capable of comparatively rapid movements when disturbed, but normally it does not seem to move very far from the end

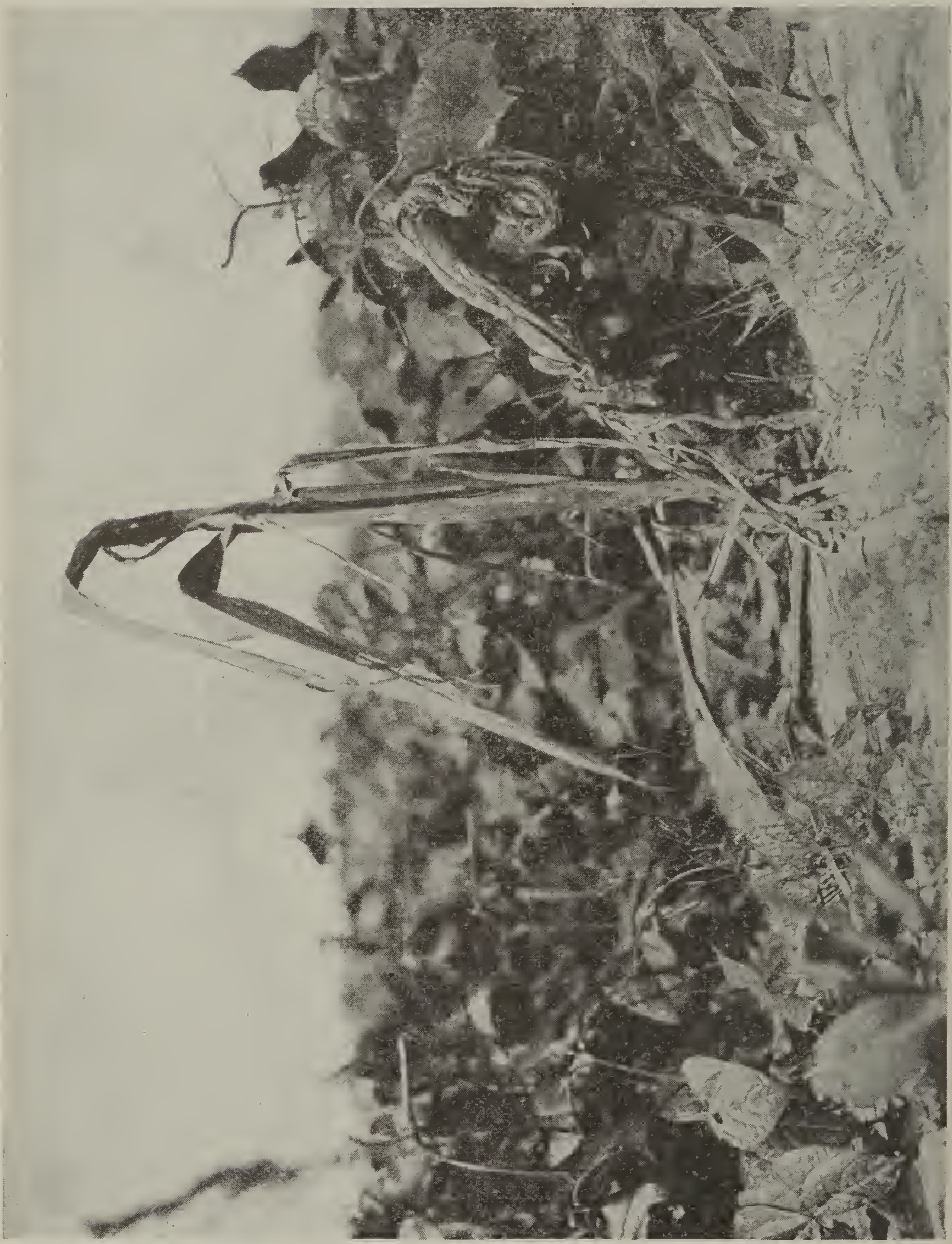


FIGURE 42. Corn plant showing characteristic stunting caused by larvæ.

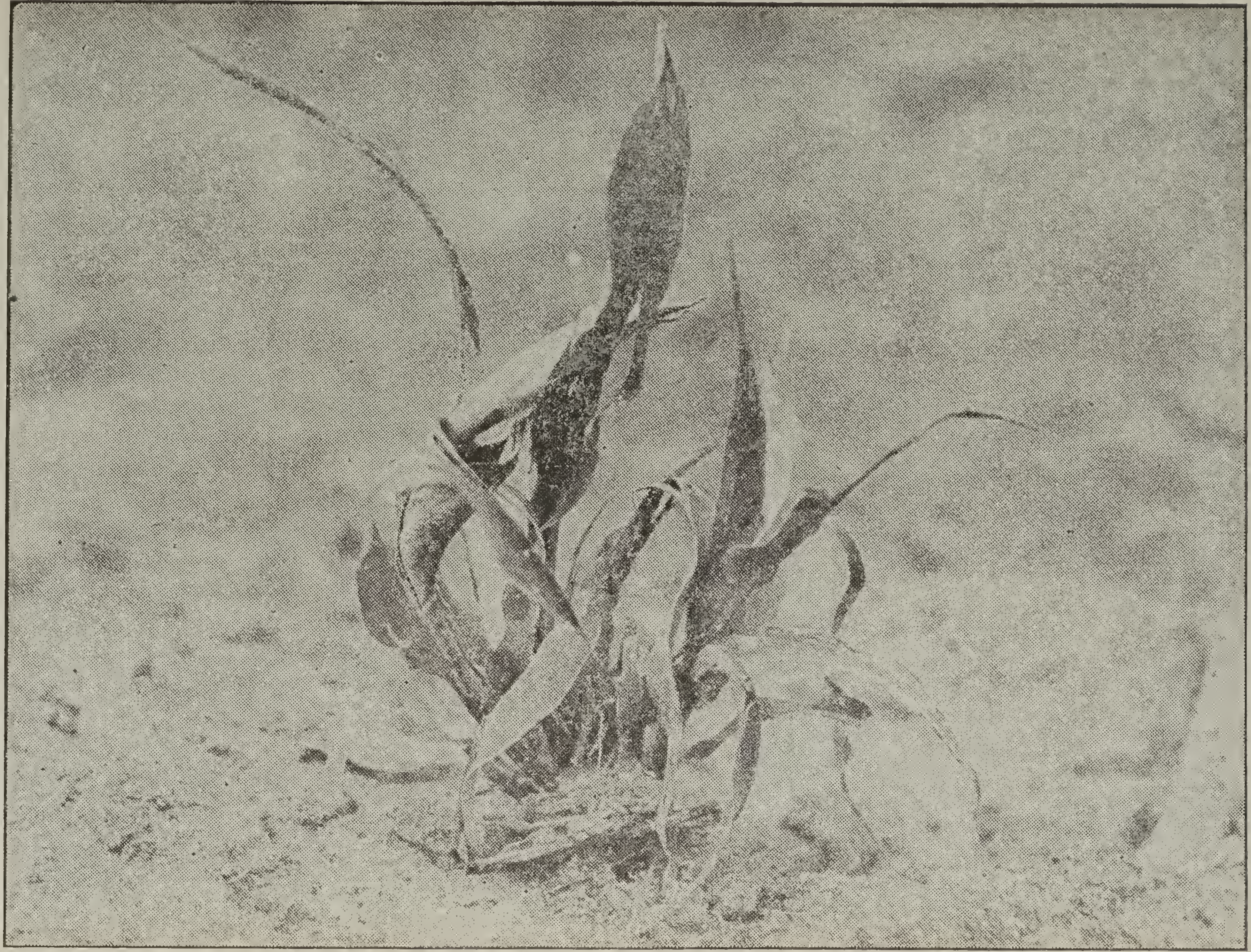


Figure 43. Corn plant showing characteristic inury caused by larvæ.

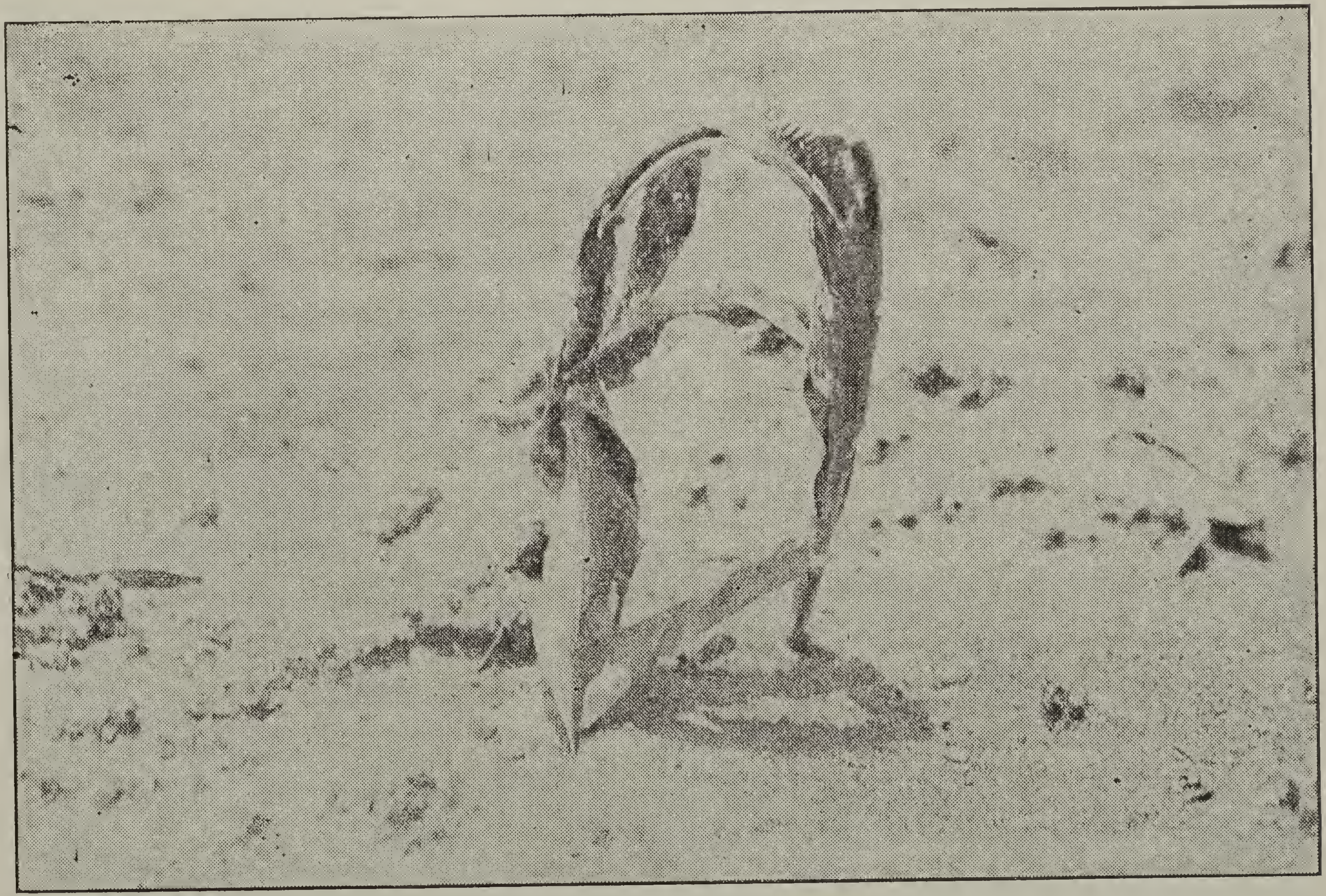


FIGURE 44. Corn plant injured by larvæ.

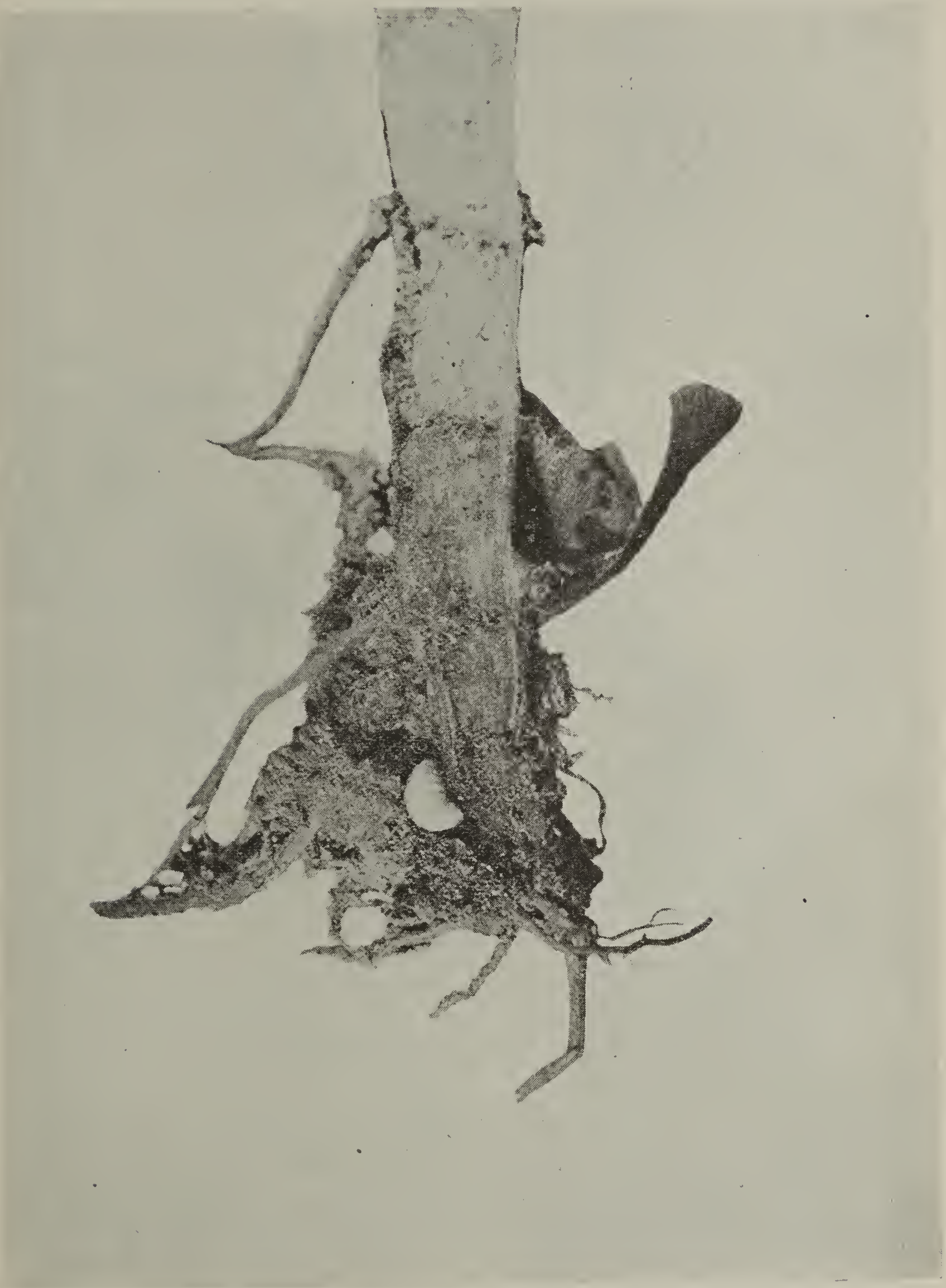


Figure 45. Larva working among the roots of corn.

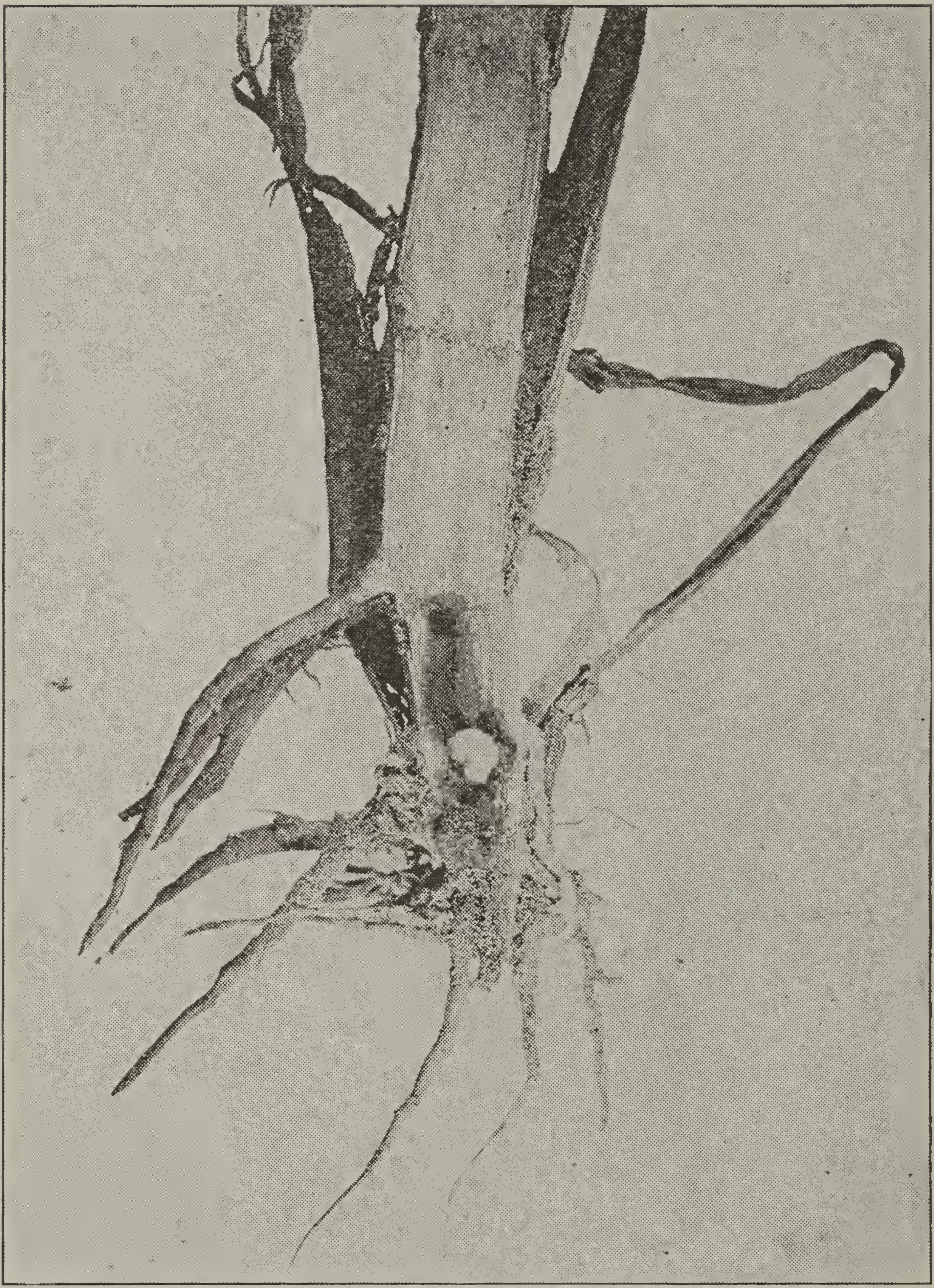


FIGURE 46. Larva in stalk of corn showing burrow.

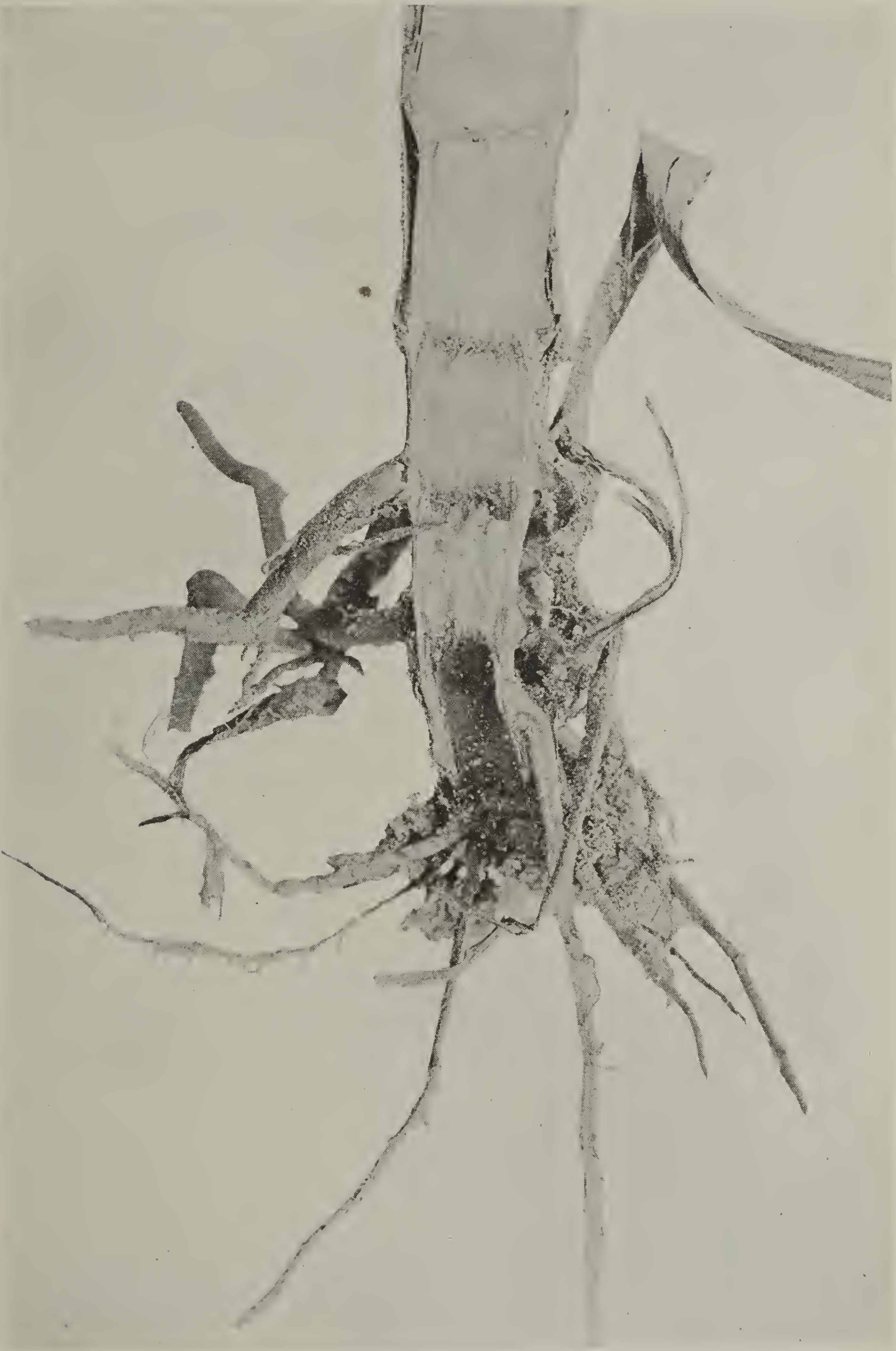


FIGURE 47. Corn plant showing injury to tap-root by larva.

of its tunnel where it is feeding. In the insectary the larvæ in the first day or two after hatching are capable of constructing an enormous length of tunnel, comparatively speaking. It is nothing unusual for one of these small larvæ less than two milimeters in length to burrow four or five times the length of a section of cornstalk seventy-five milimeters in length in a single day, or more than one hundred and fifty times its own length.

The turning and twisting habits of the prepupal stage of the larvæ is given in the discussion of the formation of the pupal cells. The larvæ as they prepare to molt have the same habit of turning about in their burrows. This most certainly helps them very much in getting rid of the old cast skin, as the larvæ usually work themselves forward at the same time they are turning; thus they travel in a spiral, and the cast skins are gradually worked backward and left eventually in the tunnel behind.

MORTALITY DURING LARVAL STAGE

In our breeding cages we have tried, in as far as possible, to give the larvæ natural conditions and at the same time make it possible to observe the larvæ from day to day. This undoubtedly subjected the larvæ to conditions which must have been in part, at least, adverse to their best development. On the other hand, larvæ which were observed every day completed their development in the same average time as larvæ which were disturbed only as often as was necessary to provide them with fresh food.

During the summer of 1915, 265 larvæ were isolated and studied for mortality data. These larvæ were treated in the ordinary way for rearing larvæ save that they were disturbed only when it was necessary to give them fresh food. The most unnatural condition introduced, so it seemed to the writer, was the fact that the pieces of cornstalks sometimes became slightly soured before they were replaced. In a few cases it seemed that natural decay, which sometimes sets in overnight in our climate, was the cause of the death of the larvæ, but other larvæ were able to withstand a condition which seemed actually identical in every respect. So it seems we are safe in concluding that the conditions were perhaps comparable with conditions in the field, and for the present, at least, we might say that no more larvæ die in the laboratory under the above conditions than would die in the field under natural conditions.

Therefore the following figures are presented for what they are worth. Of the 265 larvæ tested for this point, 138 passed through their various molts and were able to pupate; in other words, a mortality of slightly more than 45 per cent from egg to pupa.

In our regular breeding cages, where an atempt was made to secure every molt, 119 larvæ out of 276 died before they were able to pupate.

These were distributed as follows: 53 larvæ died before passing first molt, 18 larvæ before passing second molt, 14 larvæ before passing third molt, 14 larvæ before passing fourth molt, and 20 larvæ before passing fifth molt or pupating. This gives a total mortality from egg to pupa of slightly more than 43 per cent; figures in every way comparable to those above.

DESCRIPTION OF THE PUPA

Pupa stout, naked, soiled whitish in color, a typical rhynchorphorus pupa with the following minor points (Figs. 48, 49, and 50).

Ventral view: rostrum stout, reaching between the first pair of tibia, with three pairs of prominent tuberculate hairs on the base; antennæ elbowed, almost equaling the front femora; eyes not prominent; legs sub-equal in length, the hind pair covered by the wings, each femora with a stout hair near its distal end, tarsi not distinctly jointed, wings with prominent longitudinal ridges; five stout tuberculate hairs on the eighth ventral segment of the abdomen.

Lateral view: head deflexed; prothorax depressed anteriorly, usually with two short tuberculate spines, one about middle of anterior half and the other about middle of posterior half; prothracic spiracles large; wings prominent, curving ventrally between second and third pairs of legs, covering basal half of hind femora, with ten prominent longitudinal ridges; first six abdominal spiracles rather prominent, the others not evident, four long hairs on the dorsal part of the eighth abdominal segment.

Dorsal view: head nearly concealed by the prothorax; meso-thorax and meta-thorax distinct, the former terminating posteriorly in a prominent triangular lobe between the wings; the first to sixth abdominal segments nearly equal in size, with two groups of slender tuberculate marginal hairs either side of the median line, the hairs in either group about equidistant from each other, four in number; laterally there is a single tuberculate hair about mid-distant between the spiracle and the marginal group of tuberculate hairs; the seventh abdominal segment destitute of hairs save a single lateral pair; the eighth with the four pairs of hairs as described above.

SIZE OF PUPÆ

In all, twenty pupæ were measured, selected at random throughout the season. Two measurements were taken; the total length measured while the pupa was resting on a flat surface and the width across the mesothoracic legs measured from patella to patella. These measurements are given in Table XI below.

The extremes in length being 11.08 mm. and 15.74 mm., the average being 12.99 mm. The width varied from 5.08 mm. to 6.43 mm., the average being 5.69 mm.

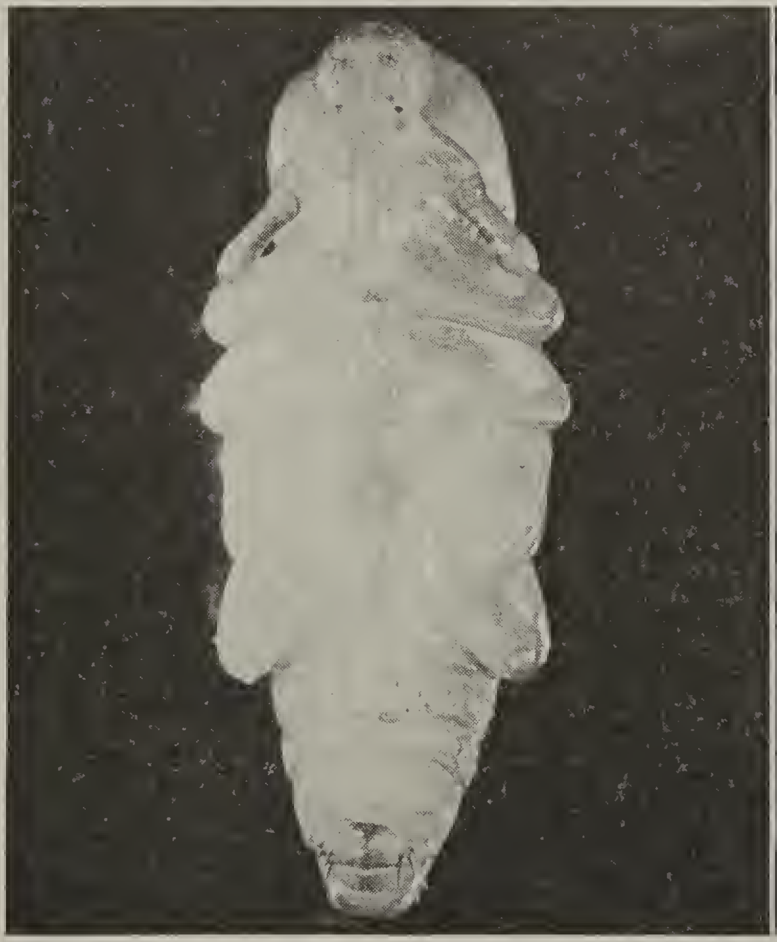
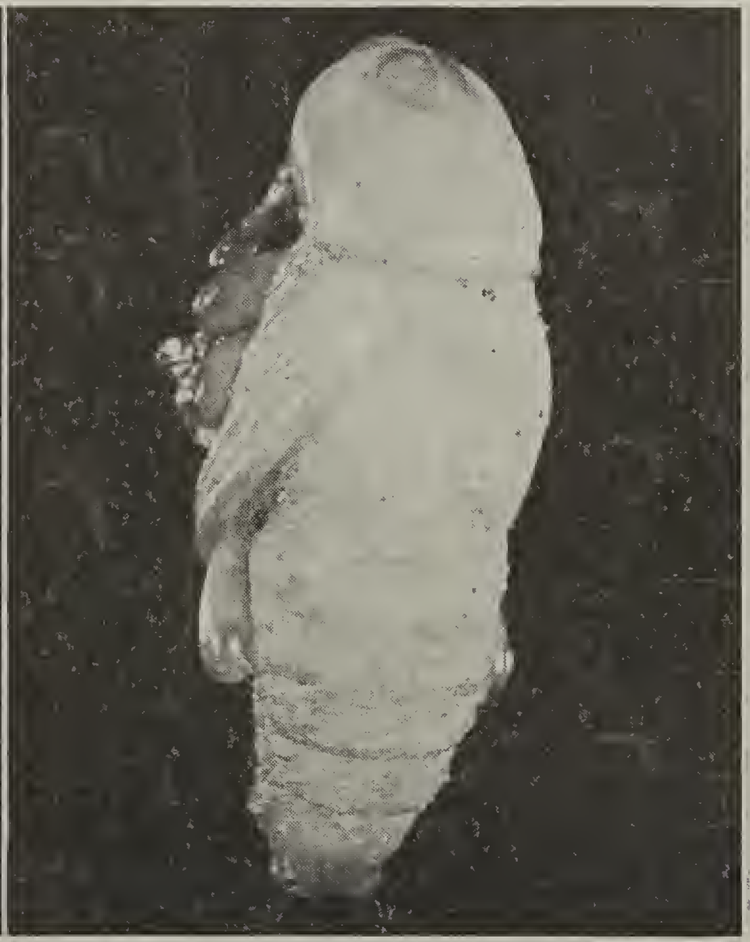
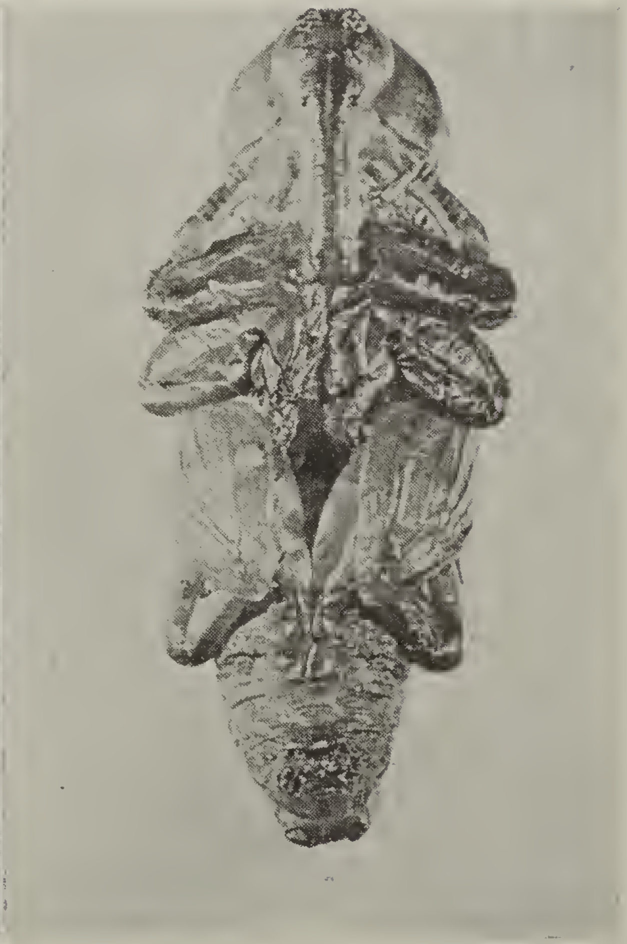




Figure 48. Young pupa, ventral view. X6. Figure 49. Dorso-lateral view of young pupa. X6. Figure 50. Old pupa, ventral view. X6.

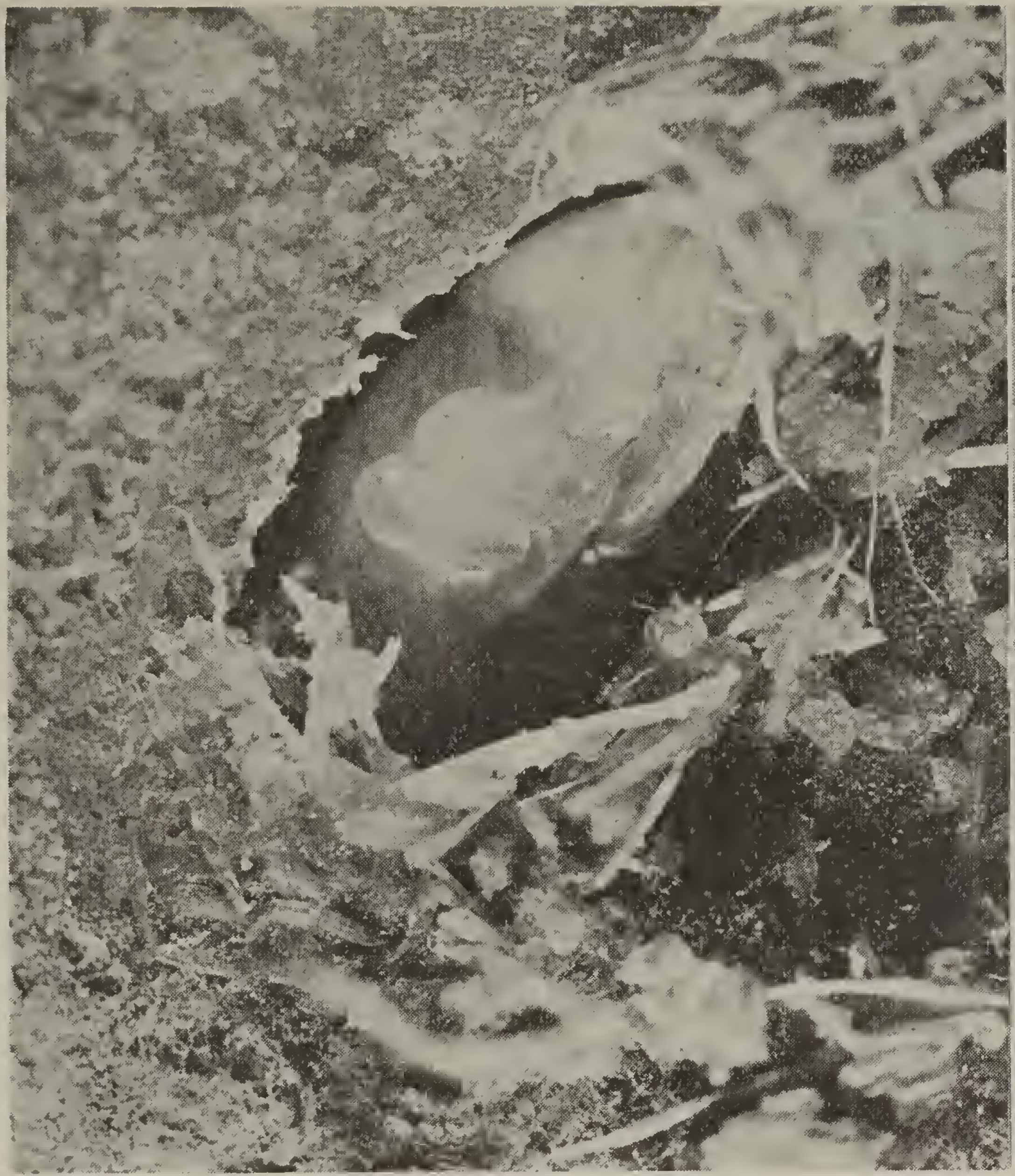


FIGURE 51. Pupa in pupal cell, lateral view. X6.

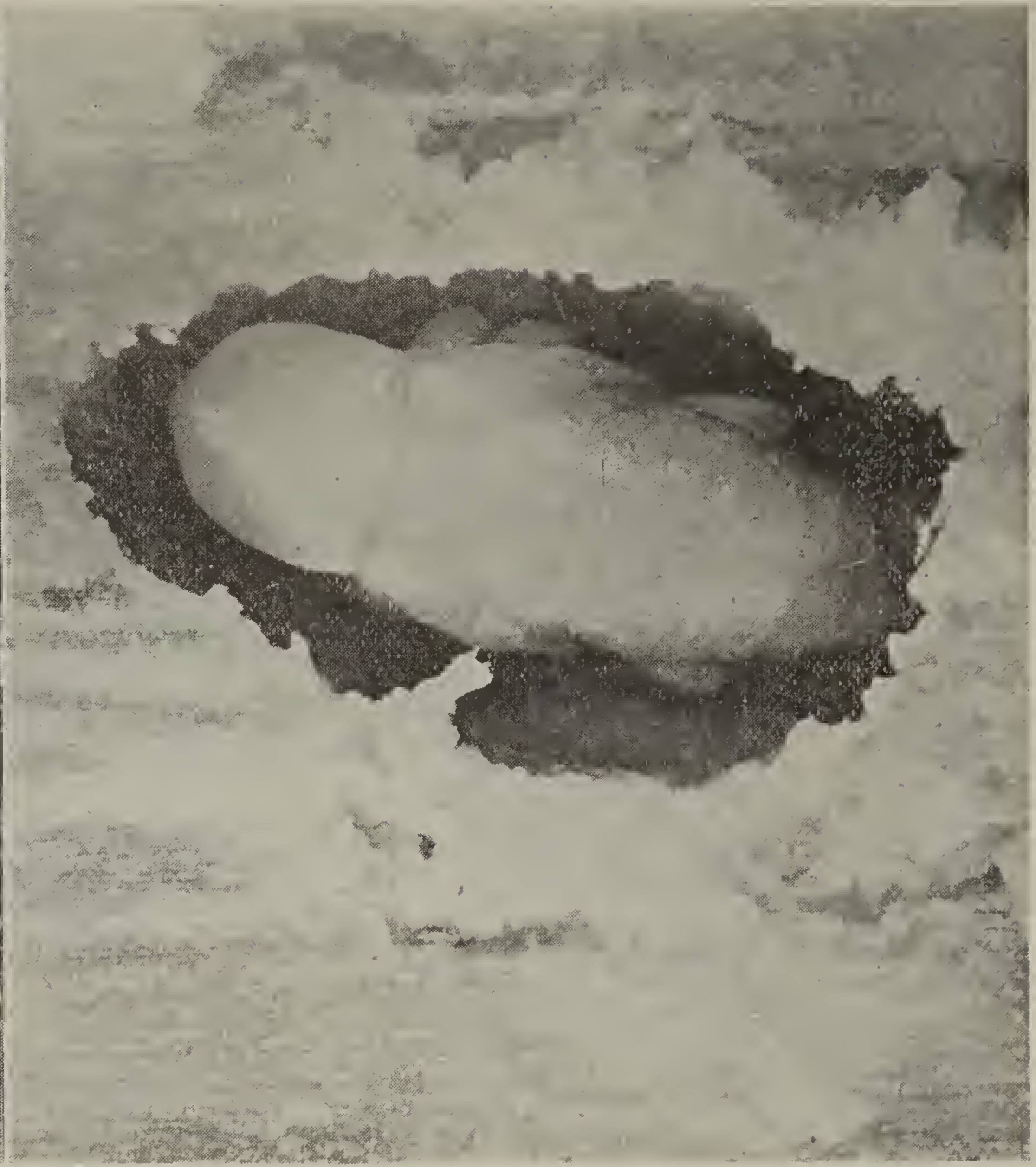


FIGURE 52. Pupa in pupal cell, dorsal view. X6.

TABLE XI.

MEASUREMENTS OF 20 PUPÆ OF THE CORN BILL BUG.

Number	Total Length in mm.	Width Across Mesothoracic Legs in mm.
1	13.87	6.17
2	15.74	6.43
3	12.26	2.56
4	13.56	6.13
5	14.39	6.04
6	12.65	5.47
7	11.39	5.26
8	12.56	6.17
9	12.56	5.56
10	11.08	5.08
11	13.39	5.95
12	14.30	6.13
13	13.56	5.65
14	11.30	5.30
15	12.95	5.86
16	12.13	5.13
17	13.48	5.26
18	13.08	6.08
19	12.95	5.30
20	12.65	5.65

PUPAL CELLS

In our work in the insectary and laboratory the larvæ when they became full grown formed a compact oblong-ovoid cell in the stalk of corn, using partly masticated pieces of the cornstalk to plug the larval burrow and compacting the whole by interminable turnings and twistings as noted above (Figs. 51, 52, 53, and 54). In these cells the pupa fit loosely.

In the field some pupal cells are formed in the stalks of corn and some are formed in earthen cells in the soil just beneath the roots, usually about one inch deeper than the hole made when we pull a stalk up by the roots (Figs. 56 and 57). These cells made in the soil are smooth and compact internally, but are unlined for the most part. Occasionally one finds a pupal cell where the tap-root has been entirely eaten away; then the pupal cell may be lined with fragments of the well masticated tap-root. But so far as our observations go, the larvæ do not move material very far with which to make the lining of their pupal cells, but take whatever comes first to hand, be it either fragments of stalk or roots or particles of soil.

EFFECTS OF MOISTURE UPON THE PUPÆ

The pupæ seem to be little affected by moisture. They can be drowned by complete immersion in water, but anything much short of complete immersion seems to have little effect upon them. Neither does the amount of moisture seem to have much effect upon the duration of the pupal stage. Pupæ kept in tight tin boxes with the air more than saturated with water emerged on the average in the same time as pupæ which were exposed to the dry air of an average room or those exposed to the average humidity out of doors in a screened insectary.

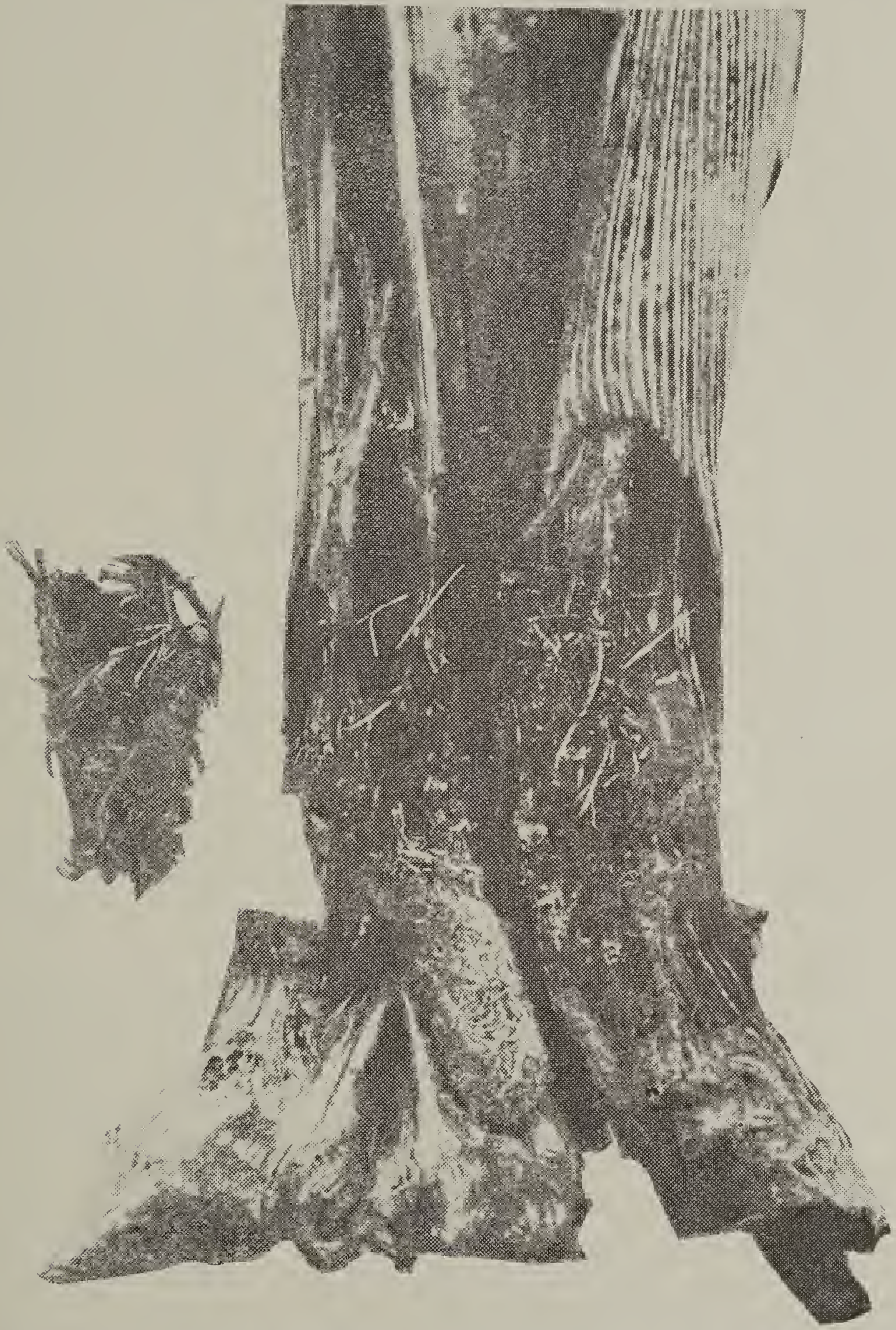


Figure 53. Pupal cells formed of corn fiber for protection of pupa; emply pupal cell on left was formed in the soil just beneath corn stalk in which the larva completed its growth. Pupal cell shown in corn stalk was in natural position at lower end of feeding cavity.

MORTALITY OF THE PUPA

Not as extensive figures have been kept on the mortality of the pupæ as upon the mortality of the larvæ. During 1915 147 pupæ were observed; from this number 110 adults were secured, giving a total mortality of slightly more than 18 per cent. Whether this is comparable to conditions as they exist in the field has not been determined. Observations made during the course of this project would lead one to believe, however, that this figure is somewhat high. The pupæ used in this experiment were not often disturbed, and frequently the stalks of corn became moldy or decay set in before they were observed again. Usually the mold or bacteria present infected the pupæ, causing their death in a majority of cases.

This condition must be wanting or very unusual in the field, for out of hundreds of pupæ observed in the field, both in cyperus and in corn, the writer has never seen a single decayed or moldy pupa. Hence it seems that we would be safe in concluding that a death rate of 18 per cent is abnormally high for pupæ.

DURATION OF THE PUPAL STAGE

In 1911 fifty-three adults were reared from pupæ whose larval stages had been followed. These all passed through the pupal stages in from eight to eleven days; the greater number maturing in nine days after they had pupated.

In 1915 one hundred and ten adults were reared from pupæ whose larval stages had been followed from the hatching of the egg. These matured in from four to twelve days; the greater number maturing in from seven to eight days.

From these figures it would seem safe to conclude that seven to nine days represent the normal length of the pupal stage; the time, perhaps, inclining somewhat toward the longer time, as is represented in Fig. 58.

As is mentioned elsewhere, variations in humidity seem to have very little effect upon the rapidity with which corn bill bugs pass through the pupal stage. The normal variations in temperature during the summer months seem to affect them only slightly, if at all, for the average time for pupæ reared in an out-of-doors insectary during July is practically the same as for pupæ reared in September.

The length of time required for larval development seems likewise to have no influence upon the time required for pupal development. In other words, the average time required for a bill bug with a total larval period of twenty to thirty days to pass through the pupal stages is so near the same as the average time required for bill bugs that have a total larval period of thirty to forty days or forty to fifty days that we seem safe in concluding that the length of the larval period has no influence whatever upon the length of the pupal period (Fig. 59).

TABLE XII.

DEVELOPMENT OF 53 PUPÆ, SEASON 1911.

Record Number	Larval Development—Number Days	Date of Pupating	Date of Emerging	Pupal Development—Number Days	Sex
1	48	July 22	July 31	9	Female
2	34	July 8	July 17	9	Female
3	34	July 8	July 16	8	Male
4	40	July 19	July 28	9	Female
5	35	July 14	July 22	8	Female
6	32	July 11	July 20	9	Male
7	29	July 9	July 18	9	Male
8	27	July 7	July 15	8	Female
9	26	July 6	July 14	8	Male
10	44	July 24	Aug. 3	9	Female
11	33	July 13	July 28	9	Female
12	36	July 16	July 24	8	Male
13	30	July 10	July 19	9	Female
14	30	July 10	July 19	9	Female
15	38	July 19	July 28	9	Male
16	35	July 19	July 28	9	Male
17	36	July 20	July 29	9	Female
18	33	July 17	July 26	9	Male
19	30	July 15	July 24	9	Female
20	26	July 12	July 21	9	Female
21	28	July 12	July 21	9	Male
22	32	July 18	July 26	8	Female
23	40	July 26	Aug. 5	10	Female
24	26	July 18	July 26	8	Female
25	47	Aug. 7	Aug. 15	8	Female
26	30	July 22	July 31	9	Female
27	42	Aug. 3	Aug. 12	9	Female
28	46	July 29	Aug. 7	9	Female
29	32	July 25	Aug. 4	10	Female
30	29	July 21	July 31	10	Female
31	33	July 25	Aug. 4	10	Female
32	30	July 22	July 31	9	Female
33	35	July 29	Aug. 7	9	Male
34	26	July 19	July 29	10	Female
35	30	July 24	Aug. 3	10	Female
36	53	Aug. 17	Aug. 26	9	Male
37	38	July 25	Aug. 3	9	Male
38	36	Aug. 1	Aug. 10	9	Male
39	28	July 26	Aug. 4	9	Male
40	27	July 25	Aug. 3	9	Male
41	37	Aug. 3	Aug. 12	9	Male
42	27	July 26	Aug. 4	9	Female
43	26	July 24	Aug. 3	10	Male
44	27	July 26	Aug. 4	9	Male
45	34	Aug. 2	Aug. 10	8	Female
46	29	July 27	Aug. 6	10	Female
47	30	July 29	Aug. 7	9	Female
48	34	Aug. 5	Aug. 14	9	Female
49	32	Aug. 6	Aug. 15	9	Female
50	26	Aug. 2	Aug. 11	9	Female
51	30	Aug. 7	Aug. 15	8	Female
52	36	Aug. 18	Aug. 27	9	Male
53	36	Sept. 12	Sept. 23	11	Female

TABLE XIII.

DEVELOPMENT OF 110 PUPÆ, SEASON OF 1915.

Record Number	Larval Development—Number Days	Date of Pupating		Date of Emerging		Pupal Development—Number Days	Sex
1	46	July	10	July	18	8	Female
2	30	July	5	July	13	8	Male
3	37	July	7	July	15	8	Male
4	38	July	13	July	19	6	Female
5	38	July	13	July	19	6	Male
6	34	July	11	July	18	7	Female
7	36	July	14	July	21	7	Male
8	30	July	9	July	17	8	Female
9	32	July	13	July	19	6	Female
10	37	July	18	July	27	9	Female
11	31	July	13	July	20	7	Female
12	37	July	19	July	26	7	Male
13	31	July	14	July	19	5	Female
14	22	July	6	July	13	7	Male
15	24	July	8	July	17	9	Male
16	29	July	14	July	24	10	Male
17	34	July	19	July	28	9	Female
18	32	July	17	July	28	11	Female
19	30	July	15	July	20	5	Male
20	26	July	11	July	17	6	Female
21	27	July	13	July	18	5	Male
22	33	July	19	July	26	7	Male
23	30	July	17	July	24	7	Male
24	29	July	17	July	24	7	Male
25	29	July	17	July	21	4	Male
26	32	July	20	July	28	8	Female
27	33	July	21	July	28	7	Male
28	26	July	14	July	21	7	Female
29	31	July	20	July	28	8	Female
30	35	July	24	July	31	7	Female
31	33	July	22	July	30	8	Male
32	32	July	21	July	29	8	Female
33	33	July	22	July	30	8	Female
34	26	July	15	July	22	7	Male
35	33	July	22	July	30	8	Female
36	37	July	27	Aug.	3	7	Female
37	31	July	21	July	29	8	Male
38	35	July	25	Aug.	2	8	Male
39	28	July	20	July	29	9	Male
40	42	Aug.	3	Aug.	7	4	Male
41	47	Aug.	8	Aug.	16	8	Male
42	32	July	24	Aug.	1	8	Female
43	37	July	30	Aug.	5	6	Female
44	45	Aug.	7	Aug.	15	8	Male
45	34	July	27	Aug.	3	7	Female
46	43	Aug.	7	Aug.	14	7	Male
47	35	July	31	Aug.	7	7	Male
48	36	Aug.	2	Aug.	10	8	Female
49	37	Aug.	3	Aug.	11	8	Female
50	27	July	24	Aug.	1	8	Male
51	38	Aug.	3	Aug.	8	5	Female
52	27	July	24	Aug.	1	8	Male
53	37	Aug.	3	Aug.	8	5	Female
54	25	July	22	Aug.	29	7	Male
55	38	Aug.	5	Aug.	13	8	Male

Record Number	Larval Development—Number Days	Date of Pupating	Date of Emerging	Pupal Development—Number Days	Sex
56	23	July 21	July 29	8	Male
57	34	Aug. 1	Aug. 8	7	Female
58	42	Aug. 10	Aug. 18	8	Male
59	27	July 26	Aug. 2	7	Female
60	34	Aug. 3	Aug. 10	7	Male
61	37	Aug. 6	Aug. 14	8	Female
62	40	Aug. 9	Aug. 16	7	Female
63	38	Aug. 7	Aug. 14	7	Male
64	34	Aug. 3	Aug. 10	7	Female
65	35	Aug. 5	Aug. 13	8	Female
66	44	Aug. 14	Aug. 27	8	Female
67	29	July 30	Aug. 5	6	Male
68	41	Aug. 12	Aug. 20	8	Female
69	31	Aug. 2	Aug. 9	7	Female
70	28	Aug. 2	Aug. 8	6	Male
71	32	Aug. 6	Aug. 13	7	Female
72	30	Aug. 4	Aug. 12	8	Male
73	26	July 31	Aug. 7	7	Female
74	24	July 30	Aug. 5	6	Male
75	35	Aug. 10	Aug. 18	8	Female
76	48	Aug. 14	Aug. 22	8	Female
77	36	Aug. 12	Aug. 20	8	Female
78	32	Aug. 8	Aug. 15	7	Male
79	30	Aug. 6	Aug. 13	7	Male
80	41	Aug. 18	Aug. 25	7	Male
81	30	Aug. 7	Aug. 14	7	Female
82	32	Aug. 9	Aug. 16	7	Female
83	31	Aug. 8	Aug. 15	7	Male
84	25	Aug. 2	Aug. 8	6	Male
85	30	Aug. 7	Aug. 14	7	Female
86	26	Aug. 6	Aug. 13	7	Male
87	29	Aug. 9	Aug. 17	8	Female
88	30	Aug. 10	Aug. 18	8	Female
89	27	Aug. 12	Aug. 20	8	Female
90	34	Aug. 19	Aug. 27	8	Female
91	28	Aug. 14	Aug. 21	7	Female
92	28	Aug. 14	Aug. 21	7	Female
93	25	Aug. 11	Aug. 18	7	Female
94	38	Aug. 25	Sept. 6	12	Female
95	27	Aug. 16	Aug. 25	9	Male
96	35	Aug. 24	Sept. 2	9	Female
97	35	Aug. 24	Sept. 2	9	Male
98	35	Aug. 24	Sept. 2	9	Female
99	30	Aug. 21	Aug. 30	9	Female
100	29	Aug. 20	Aug. 29	9	Female
101	31	Aug. 23	Sept. 2	10	Female
102	30	Aug. 21	Aug. 29	8	Female
103	23	Aug. 16	Aug. 25	9	Male
104	34	Aug. 29	Sept. 7	9	Female
105	31	Aug. 26	Sept. 6	11	Female
106	34	Aug. 29	Sept. 8	10	Female
107	28	Aug. 23	Sept. 1	9	Male
108	22	Aug. 18	Aug. 29	11	Male
109	30	Aug. 26	Sept. 6	11	Female
110	26	Aug. 22	Aug. 31	9	Female

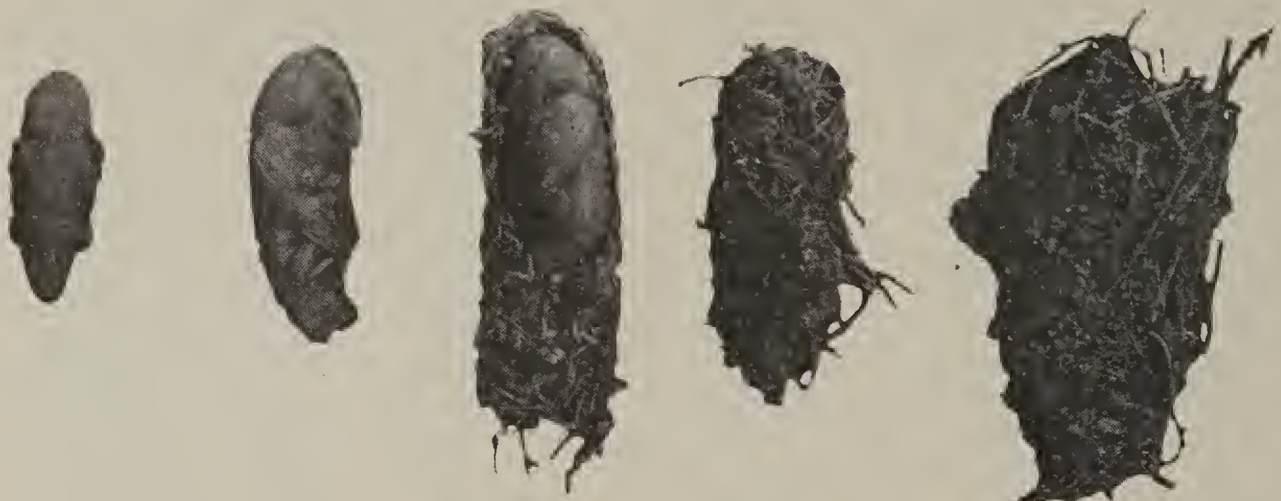


FIGURE 54. Pupæ and pupal cell formed of corn fiber; two small pupæ on left dorsal and lateral views; on right live pupa in its pupal cell. X2

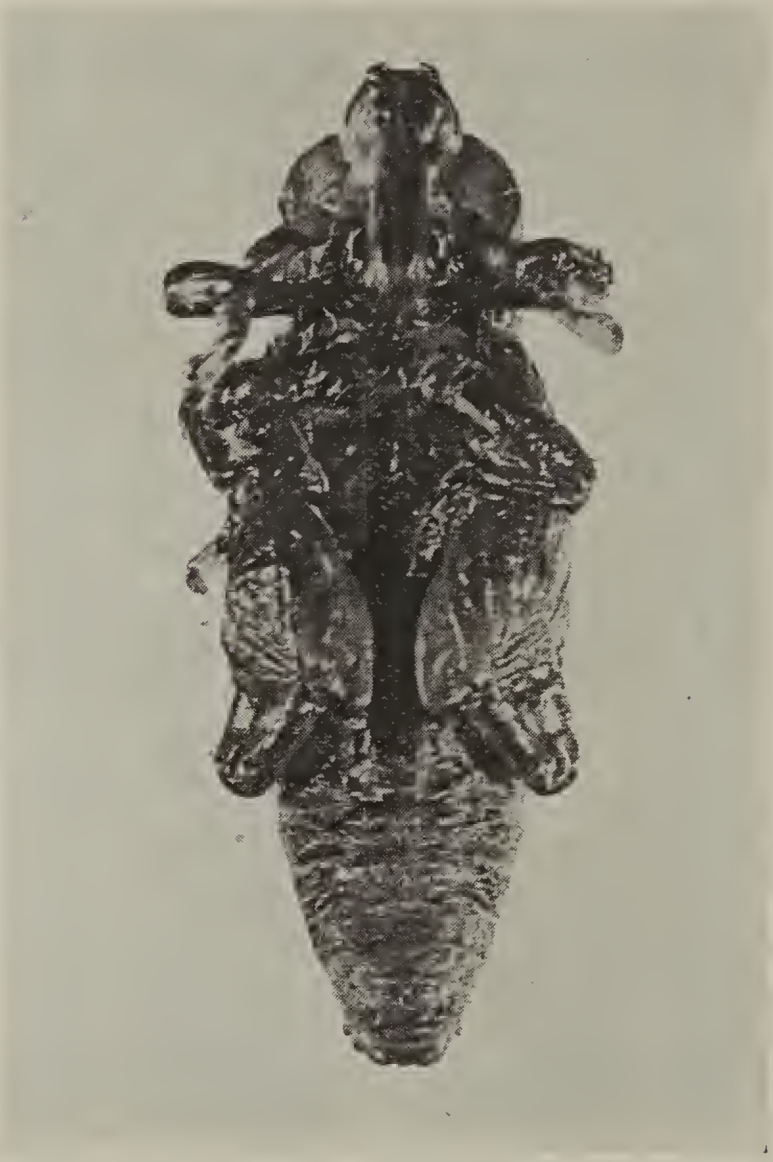


FIGURE 55. Adult emerging from pupa, only the prothoracic legs and the beak are free. X6.

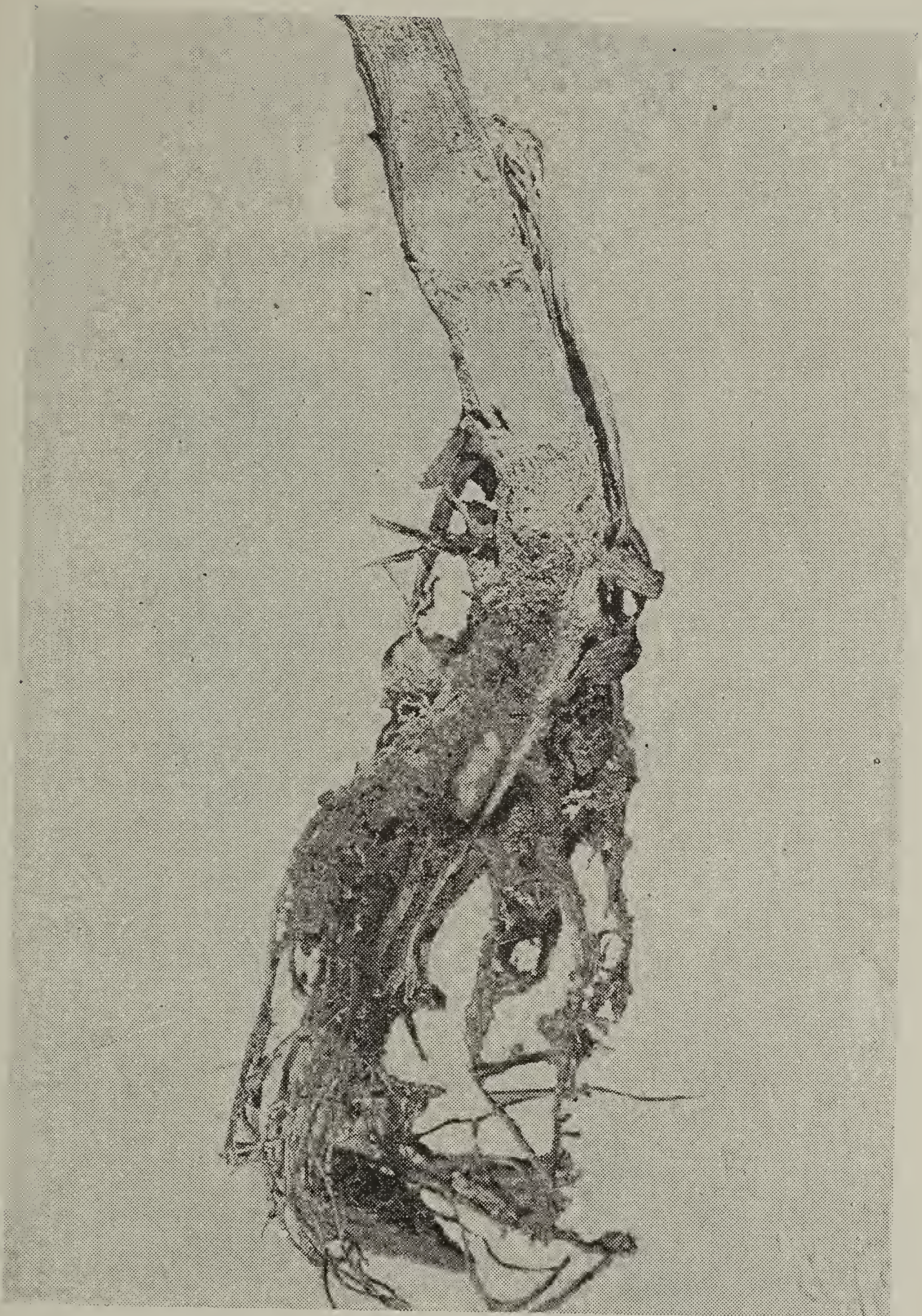


Figure 56. Pupa in pupal cell in tap-root. X1.

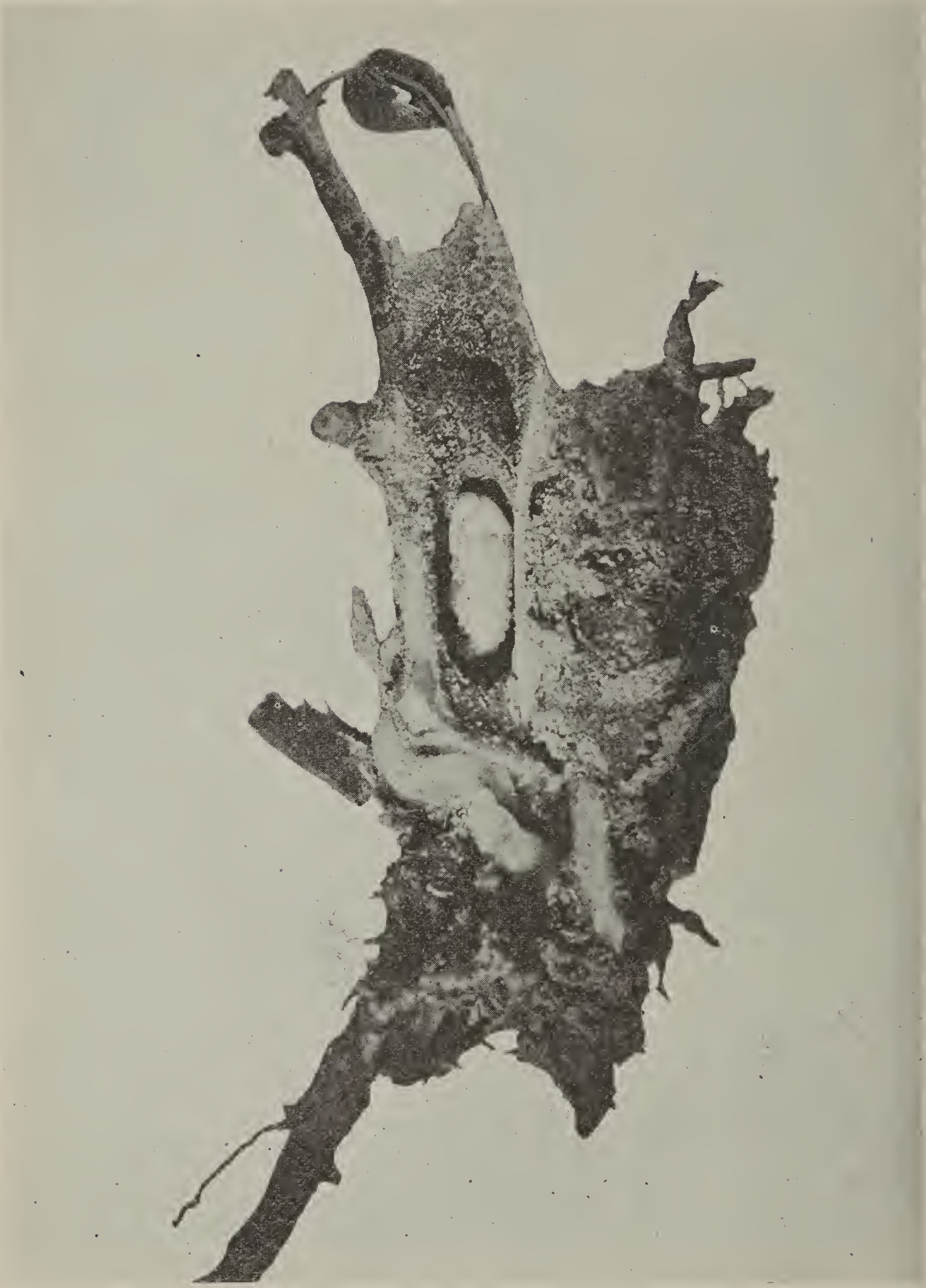


FIGURE 57. Pupa in pupal cell in ground among roots of corn stalk. X2.

TRANSFORMATION OF PUPA

The Molting of the Last Larval Skin

Usually about forty-eight hours before a larva is ready to transform to a pupa it ceases to feed, assumes a straight, rigid form (Fig. 29), and commences to turn over and over inside of the cell. This seems to compact the material with which the cell is lined and at the same time smooths the interior of the cell. This stage may be known as the prepupal stage, and larvæ usually assume this stage about forty-eight hours before they are ready to pupate. Cases have been observed, however, where the prepupal stage was assumed three days before pupation; in other cases, however, this stage is not assumed until the last day before pupation.

Just before pupation actually takes place the perfectly formed pupa may be distinguished through the transparent larval chitin. The larval chitin splits first along the epicranial and frontal sutures, and the prothorax is the first part of the pupa to emerge clearly from the cast skin, due to the fact that the head of the pupa is bent ventrally. Gradually the cast skin splits more and more posteriorly and is worked away from the body by the pupa turning and twisting its abdomen about (Fig. 31). The larval cast skin covering the legs and beak is the last to be freed from the pupa, this part of the cast skin frequently clinging to the pupa for several days. Usually the whole transformation from prepupa to pupa takes place in thirty minutes, sometimes, however, it takes longer than this. In one case observed the splitting of the larval cast skin commenced at 6 in the evening but the pupa did not have its abdomen free from the larval cast skin until the next day between 9 and 10 in the morning. Such occurrences must be rare, however, for the only other case observed, where it required the pupa longer than an hour or two to free itself from the larval skin, the pupa died before it was able to free itself, indicating that it was not normal.

The pupa is at first a pure translucent white, but in the course of a few hours it turns a pale straw yellow and retains this color until the developing adult's dark colored body can be seen through the translucent pupal skin (Figs. 48-50). This color can usually be noticed a day or two before the adult is ready to emerge.

HABITS OF THE PUPA

The pupa is naturally confined to its pupal cell. Therefore it has no habits that are especially interesting. When the pupa is disturbed it bends its abdomen first dorsally, then ventrally, and is thus able to rotate its body. It does not usually keep this process up very long before it relaxes. Usually the period of relaxation is approximately as long as the period of action which has just preceded. In most cases it takes a very violent disturbance to make the pupa become active before the period of relaxation has been of approximately the same duration as the period of activity. These alternate periods of activity and relaxation seem to follow each other in fairly regular succession over long periods of time. I do not mean to imply by the above statements that there is any accurate mechanism which regulates the periods of relaxation with those of activity, but repeated observations showed that it took a much more violent stimulus to cause activity in a pupa that had recently relaxed than it did in a pupa that had been relaxed for some time. And apparently the longer the period of relaxation, the smaller the stimulus required to produce activity.

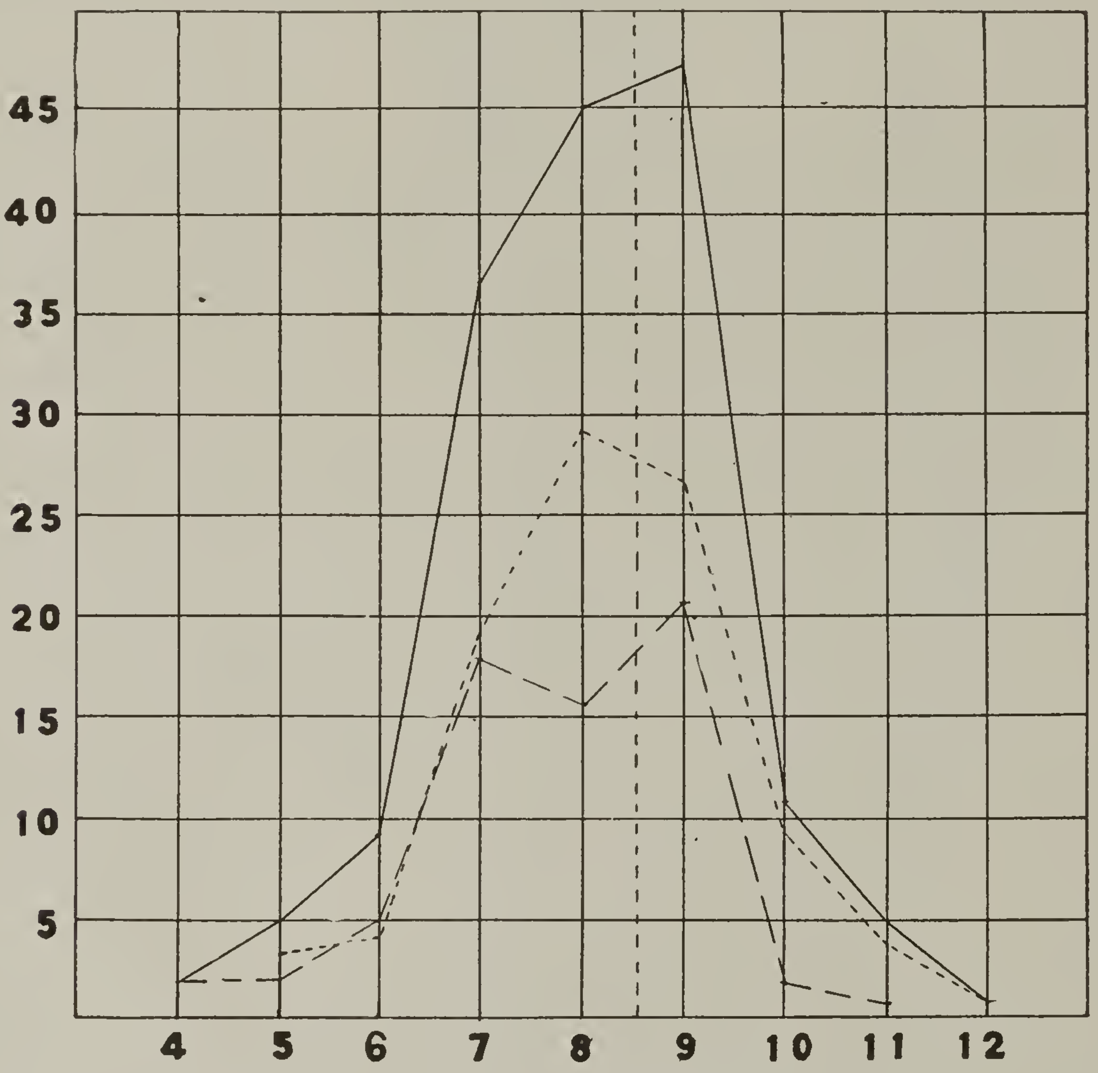


FIGURE 58. Curve to show duration of pupal stage. Dotted line (. . . .) females. Dashed line (— — —) males. Solid line (———) totals.

DESCRIPTION OF THE ADULT

The following description of the adult is summarized from Blatchley and Leng (1916), our most recent authorities on this group:

Tibiæ all broadly rounded at outer apical angle. Third joint of hind tarsi feebly dilated. Third joint of other tarsi not dilated, entirely glabrous beneath. Elytral intervals all flat, no single one of them in part or wholly distinctly elevated above the others. Beak curved, slender. Front tibiæ straight. Thorax with distinct smooth spaces. Median thoracic smooth area dilated at middle. Surface densely coated.

Oval robust. Black, densely clothed with a brownish or olivaceous clayey coating; antennæ and tarsi reddish-brown. Beak two-thirds the

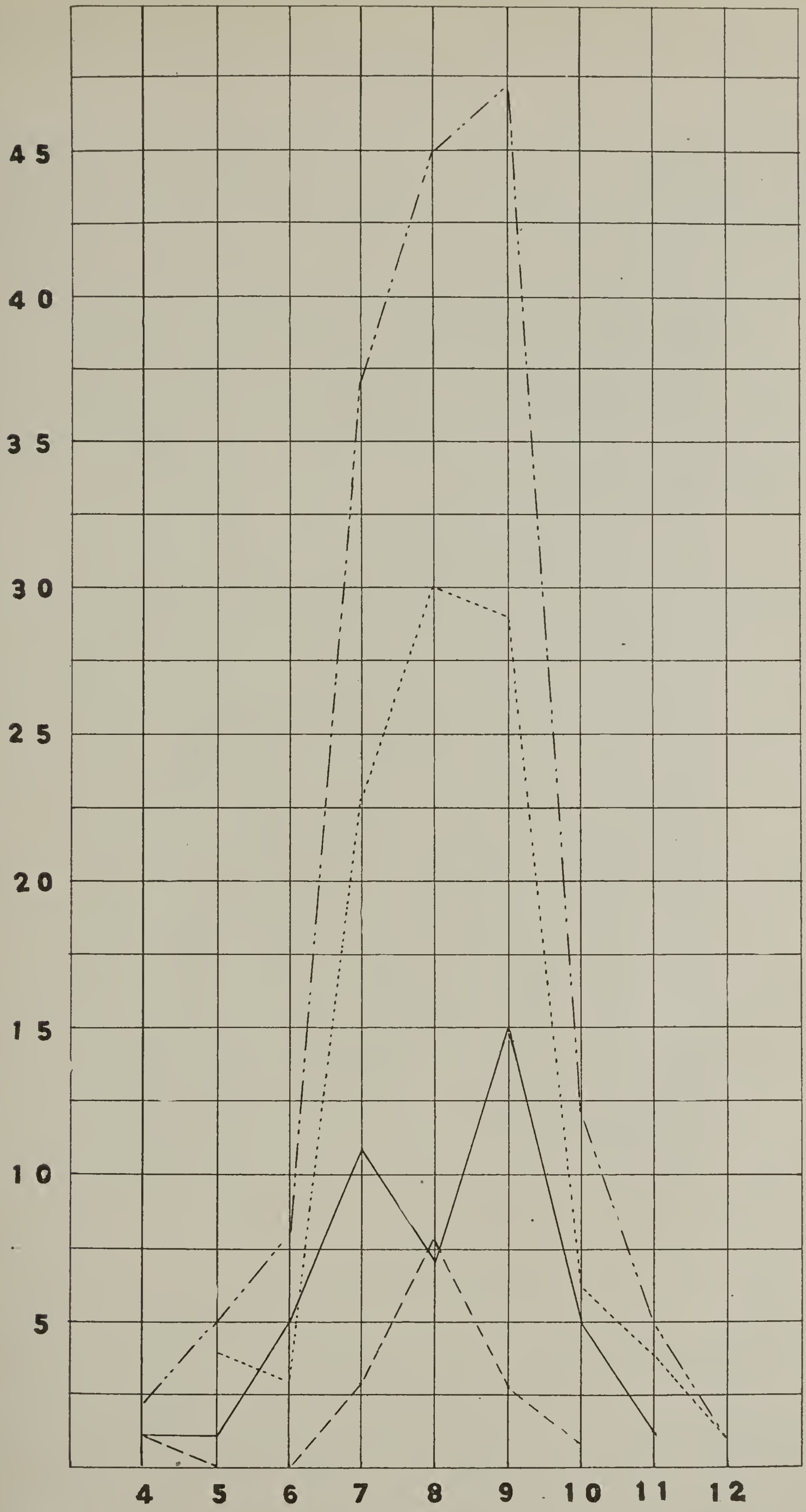


FIGURE 59. Curve to show the relation between duration of larval stage and duration of pupal stage. Solid line (————), pupæ whose larvæ required from twenty to thirty days to complete their development; dotted line (........) pupæ whose larvæ required from thirty to forty days to complete their development; dashed line (— — — —) pupæ whose larvæ required from forty to fifty days to complete their development; dashed and dotted line (—..—..—) totals for all pupæ observed.

length of thorax, compressed and sparsely punctate except near base, where it is swollen, coarsely punctate, and shallowly grooved above. Thorax slightly longer than wide, sides parallel from base for three-fourths their length, then strongly rounded to the constricted apex; disc very coarsely and irregularly punctate, the median vitta usually broadly dilated at middle, its apical portion very narrow; lateral vittæ broad at base and with a short oblique branch, their front portion often replaced by coarse punctures. Elytra oval, their surface uneven, sides gradually narrowed from humeri to apex; striæ fine, coarsely and distinctly punctate; intervals flat, their punctures hidden, and third sometimes feebly base. Humeral umbone and sub-apical callus somewhat prominent, often shining.

DURATION OF LIFE OF THE ADULT

The adult beetles found in any corn-field during the spring and early summer must, for the most part, be adults that matured the previous June, July, August, September, and October, as the adults live over the winter. (See discussion of hibernation of adults.) Laboratory experiments seem to indicate that occasionally, at least, adults might be able to live over two winters. We have never been able to keep adults through two winters, due, perhaps, to the fact that conditions are not normal; but we have been able to keep them through one winter and through the next winter until the first of March.

These beetles were collected June 20, 1910, and represented the 1909 generation, and must have matured the previous June, July, August, September, or October. They were, therefore, from seventeen to twenty-one months old when they died. These beetles might have lived longer than March 1, 1911, if they had not been disturbed during January and February to see whether they were alive. However, a miscellaneous lot of beetles hatched during the summer of 1912 and used for egg-laying records during 1913 all died before the spring of 1914, in spite of the fact that they were not disturbed. A similar fate awaited those adults reared during the summer of 1913. At the present writing, November 26, 1915, five out of the 1914 generation are still alive. In June, 1913, about 500 adults were marked in various ways to see whether any of these adults which undoubtedly represented those emerging during the summer of 1912 would be able to pass through the winter. None of these marked beetles were recovered during 1914. But in spite of this fact the writer believes that occasionally a beetle might be able to live through the winter. At least they are able to go into hibernation, and it seems only reasonable to suppose that occasionally they are able to emerge from hibernation, although at the present time we have no evidence to support this conclusion. These might represent the adults which lay only a few eggs during the summer (see egg-laying records above). At least, we have not been able to assign any other cause to the failure of certain adults to lay anything that even approximates the average number of eggs laid by a majority of the females. On the other hand, the adult females that lay only a few eggs might be females that matured early the previous summer and laid a portion of their eggs during the previous season.

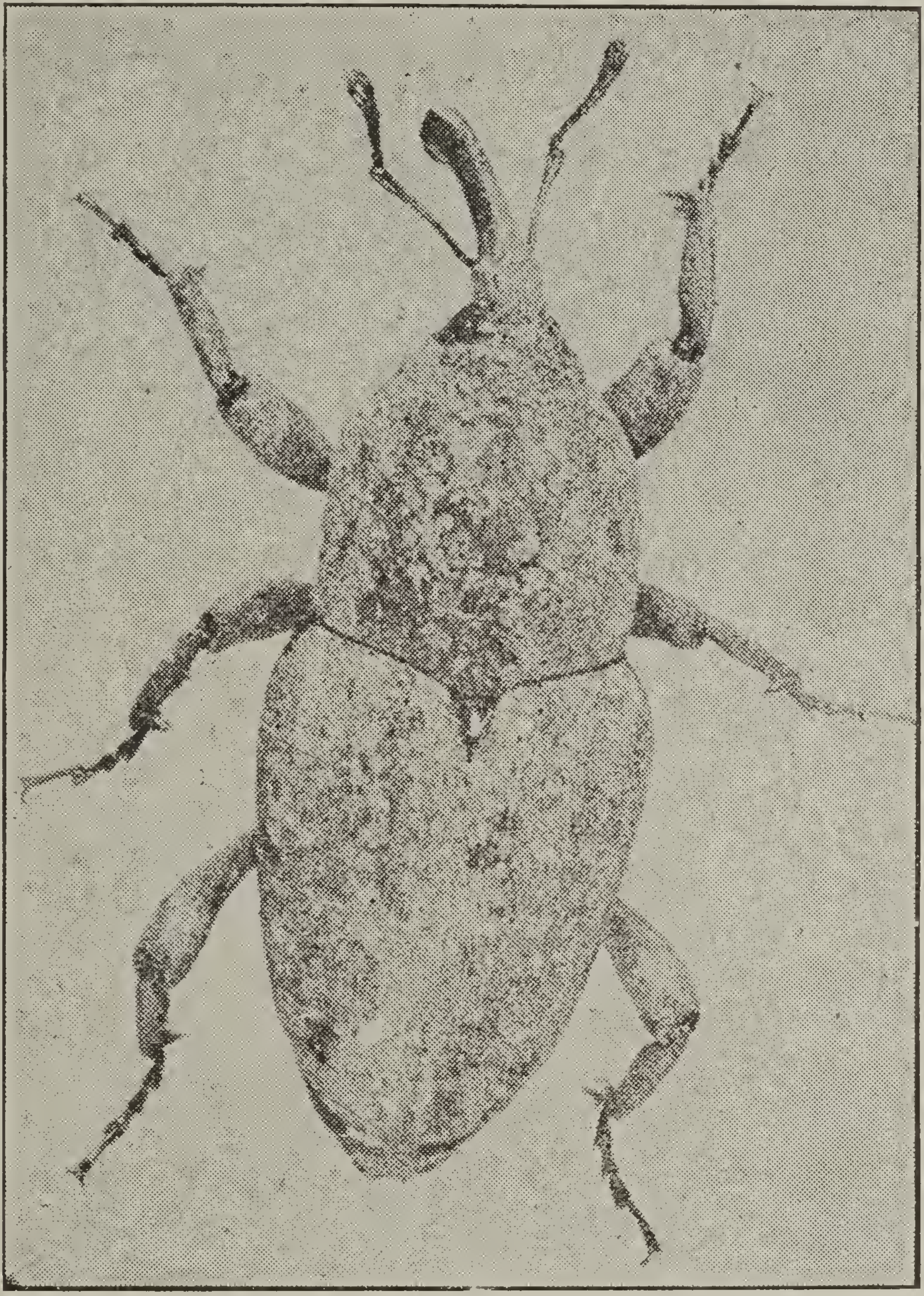


FIGURE 60. Adult dorsal view. X8.

PROPORTION OF SEXES

Of the 163 individuals that have been reared from egg to adult, 95 individuals or slightly more than 58 per cent were females, while 68 or a little less than 42 per cent were males.

In the field the proportion of sexes seems to be more nearly equal. As a usual thing, active females are attended by males, and inactive beetles under stones, sticks, or clods of dirt are usually equally males and females. In July, 1912, this point was made a special point for investigation, and in all 627 individuals were counted and so marked as to prevent their being recounted. It is believed that this represents practically all of the bill bugs present on a plat of one-third acre. Of 627 individuals, 329 or slightly more than 52 per cent were females; while 298 or slightly less than 48 per cent were males. In addition to this, many other observations and the counting of smaller numbers of bill bugs would indicate that the proportion of sexes in the field was approximately equal. The table below will indicate the approximate numbers of bill bugs and their distribution as determined from counting all the bill bugs that could be found on a plat about one-eighth of an acre in extent as counted June 10, 1915, between 10 a. m. and 3 p. m. In this case over 60 per cent were males and less than 40 per cent were females. And while it is possible that some bill bugs may have been missed, an effort was made to secure all of the bill bugs present, and certainly the vast majority of the bill bugs present on that date were recovered and counted. This incident is merely cited as one of the incidents that force us to the conclusion that the proportion of sexes must be nearly equal under ordinary conditions, in spite of the fact that in the total numbers counted from all sources there are 110 females to every 100 males.

TABLE XIV.

SHOWING THE DISTRIBUTION OF ADULT BILL BUGS ON JUNE 10, 1915. PLOT A—ONE-EIGHTH OF AN ACRE.

	Females	Males
Feeding	2	2
Egg laying	1	0
Actively crawling about	0	1
Under clods, sticks, etc.	24	39
Totals	27	42
Per cent	40	60

APPEARANCE OF THE ADULTS IN THE SPRING

In the eastern part of the State the adults appear in the corn-fields as early as mid-April. Their numbers increasing gradually, as the weather grows warmer, so that usually by the first of June all the over-wintering beetles seemed to have emerged from hibernation, and usually towards the end of June their numbers seem to be increased by newly transformed adults which may be readily distinguished at the time of their emergence by their velvety brown color. The numbers of adults increase gradually during July and the first half of August. The bill bugs usually completely disappear in fields of corn planted in April and May by the latter part of August. These bugs seem to locate in later planted corn if any be available, but just where they go if corn is not available has never been definitely determined. Perhaps they feed upon some of the wild food plants, but close observation fails to show any especial increase in their numbers in such situations. Neither can any be found in the usual hibernation situations save young beetles which have evidently not yet emerged from their pupal cells.

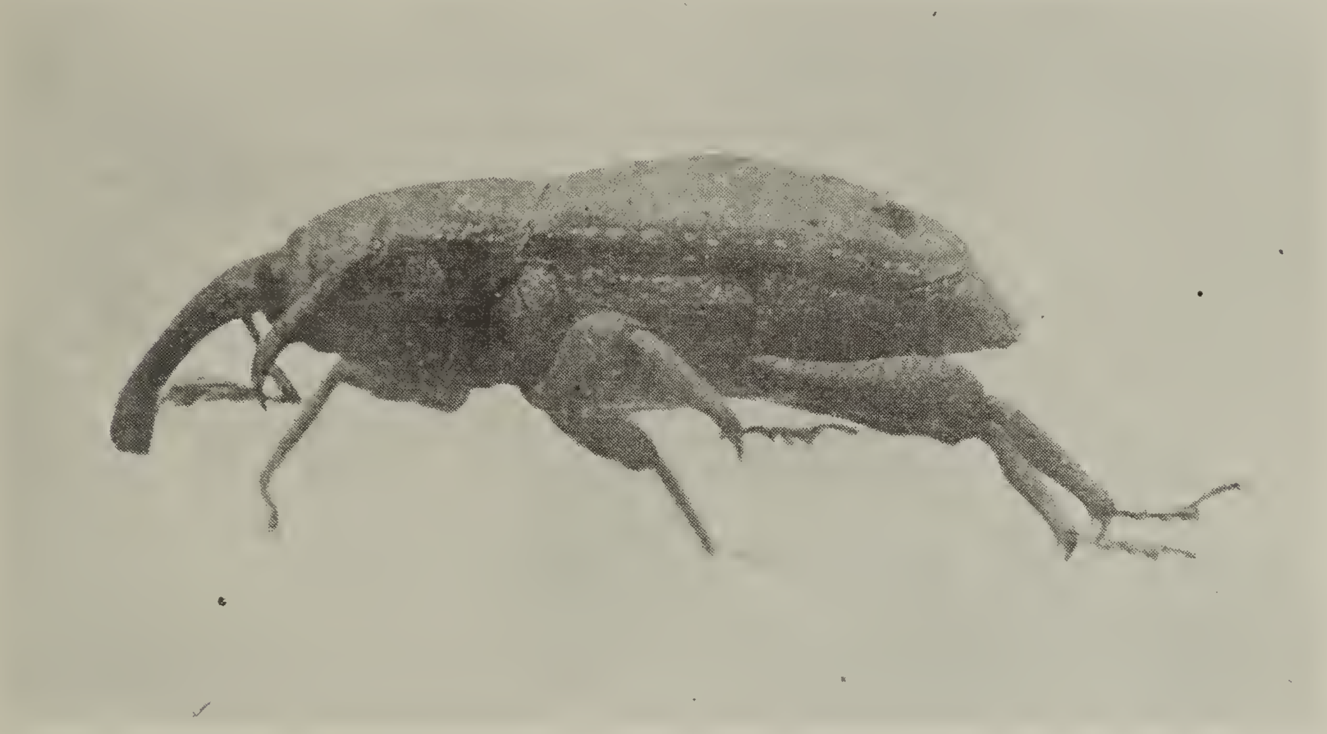


FIGURE 61. Adult lateral view. X6.

DIURNAL ACTIVITY

The diurnal activity of corn bill bugs in the field was made a special point for investigation during the summer of 1912, and since that time many additional observations have been made which support the conclusions drawn at that time. In making these observations, 627 adults, 329 of which were females and 298 were males, were marked in various ways and studied throughout the month of July and part of August. These adults, it is believed, represented all of the individuals present on a plat of about one-third of an acre in extent. Observations were made at various times for every hour of the day and night. From these observations we have concluded that there are two main periods of activity for the corn bill bug, the activities for the most part consisting of feeding and egg laying for the female and feeding and mating for the males. For a fuller discussion of these activities, see habits of adults below. Two main daily periods of activity are followed by two periods of inactivity, when the bill bugs crawl away under any shelter that may present itself, such as clods, partially buried sticks or pieces of cornstalks and stones. The activities vary somewhat from month to month and from day to day. But the activities on a cool, cloudy day late in the season approximate the activities on a warm, bright day early in the season. I have attempted to reduce all of these observations to the form of a curve (Fig. 62).

In order that all of the observations made during the summer of 1912 and during the following summers might be correlated, the ob-

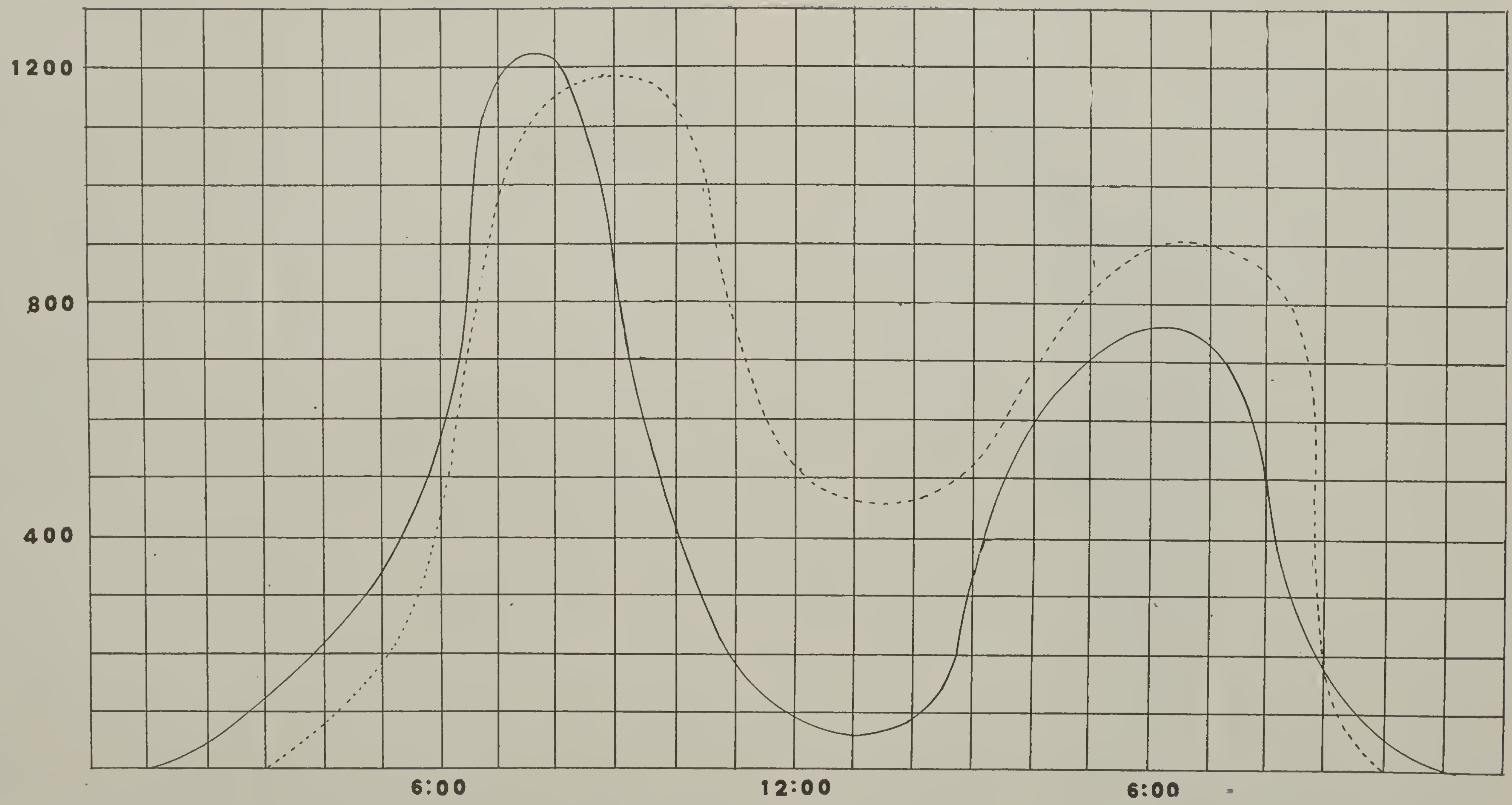


FIGURE 62. Curve to show diurnal activity based on counts as made in the field. Solid line (————) to represent activity from mid to late season. Dashed line (— — — —) to represent activity early in the season. On the basis of hundreds active per acre.

servations on the diurnal activities for bill bugs have been reduced to a curve representing the numbers of bill bugs active per acre. This curve does not take into consideration the number of bill bugs that were not active, as it was impossible to be absolutely sure whether you had actually secured all of the inactive bugs lying under clods and in similar situations. On the other hand, it was usually a comparatively simple matter to determine the number of bugs which were feeding, laying eggs, or otherwise engaged in any of the daily activities of adult corn bill bugs.

On the plat where most of the observations were made during the summer of 1912, 627 adult corn bill bugs of both sexes were found. This plat represented about one-third of an acre. Thus we have bill bugs occurring at the rate of more than 1,800 per acre. Yet in spite of this fact, the average rate per acre at the period of greatest activity, about 8 a. m., was usually not more than 1,200 per acre. Some other observations made in 1913 confirmed this general conclusion, that is, that even at the period of greatest activity only about two-thirds of the bill bugs in a given area would be actively engaged in feeding or egg laying.

From an inspection of Fig. 62 it will be observed that on the average bright day in mid-season the two main periods of activity are from 6 to 9 a. m. and from 4 to 8 p. m. The main periods of inactivity are from 10 a. m. to 3 p. m. and again from 10 p. m. to 3 a. m. Ordinarily on bright days no bill bugs will be found active from 11 p. m. to after 2 a. m. The first bill bugs become active between 2 and 3 a. m., these being joined by others between 3 and 4. a. m. So that on the average there are about 200 active bill bugs per acre by 4 a. m. Usually by 5 a. m. another 100 per acre have joined those already at work; by 6 a. m. the number of active bill bugs per acre has usually increased to 600, and by 7 o'clock this has usually doubled again; so that there are on the average about 1,200 bill bugs per acre actively engaged in feeding and egg laying. By this time some of the earlier ones to become active have begun to seek shelter, but usually there are enough more becoming active, so that up to 8 o'clock the numbers are about constant. Very few new ones join the ranks after 8 o'clock, so that the numbers drop off rather rapidly, and late in the season, during late July and early August, on clear days when the sun is very hot in the corn-fields practically all of the bill bugs will be under cover by 11 a. m., where practically all will remain until about 2 p. m. In mid-season, mid-June to mid-July, the later arrivals in the morning will linger through the warmer part of the day, so that it is not unusual to find bill bugs at the rate of 200 to 300 per acre at this time of the day.

Still earlier in the season, as from mid-May to mid-June, and on cloudy days later in the season, the bill bugs do not become very active until about 6 o'clock in the morning, but by this time there are usually at the rate of 500 bill bugs per acre actively at work in the field. This number increases to nearly 1,200 per acre by 8 o'clock; this number remaining practically constant until 10 o'clock, when the number actively at work usually falls off rather rapidly to about 500 per acre at 12 o'clock. This number remains practically constant until about 3 o'clock, when the numbers commence to increase gradually up to from 900 to 1,000 by 5 o'clock. This number remains practically constant until 7 o'clock, when the number decreases very rapidly, so that by 9 o'clock practically every bill bug in the field has ceased his activities for the day, and if the weather remains cloudy these activities are not resumed until the following morning between 3 and 4, when a few of the earliest to cease their activities then again become active for the new day.

HABITS OF THE ADULTS

The egg-laying habits of adults are touched upon in another connection (see methods of oviposition below) and the general daily activities of adult bill bugs have been discussed under the heading of diurnal activities. However, the general feeding, mating, and other habits have not been discussed.

In discussing the habits of bill bugs one is almost forced to use terms which have long been used to denote conscious human actions. The writer has no intention of implying that either the stimulus or the reaction is the same as it would be in human beings, to produce the results described. He has merely used these terms because they happen to give the clearest picture of the reactions of bill bugs to certain complexes of stimuli.

The adults feed at or near the level of the ground, tearing away the outer leaves of the plant so as to be able to get at the tender growing leaves or bud of the plant. In feeding, the bill bug always assumes a position with head downward. When the plant is young the bill bug may feed at a distance of an inch or two above the surface of the soil, but usually they have the head and thorax below the surface and not infrequently practically the whole body will be below the surface. Occasionally the adults are so completely buried that their presence is not suspected until the plant is pulled up, when the adult will be found clinging to the portion of the stem below ground. Usually, however, a portion of the pygidium is left exposed, so that close observation will reveal the presence of the adult. This, however, requires very close inspection, for, as explained above, the color of the bill bug very closely approximates the soil in which it is living, due to the fact that small particles of the soil adhere to the body.

The bill bug is not easy to dislodge from the corn plant while it is feeding. When disturbed it clings to the stalk of corn by means of the tibial spines, so that usually part of the stalk of corn is torn away before the bill bug is removed.

In its movements the bill bug is very slow and deliberate, evidently depending upon its color and slow gait for protection. At times the adult moves rather rapidly, but this gait is not long continued, and usually after each period of rapid movement it will remain perfectly motionless for a period usually equaling if not exceeding the period of rapid movements. This jerky gait always impresses one with the thought that the bill bug fully expects every movement it makes to betray it to its enemies. Their total movements thus become so slow as to exhaust the patience of any human being who is observing them in the hope that he will get a composite picture of their activities. The same slowness of gait applies to all of their activities, be it mating, egg laying, feeding, or what not. All of the movements of the bill bug seem to be entirely aimless and without any definite purpose, or such is my conclusion, after watching them for the past several seasons both in the field and in the insectary. In the field bill bugs on coming from their retreats under clods or sticks will proceed in a jerky manner often for a distance of many feet, often passing in their wanderings within a fraction of an inch of stalks of corn, to bring up eventually at a stalk that is, so far as one can observe, not better in any way than stalks that have just been passed. Frequently they will take such journeys of exploration, sometimes wandering across a plat of corn thirty or more feet in direct line from the starting point, only to turn in an aimless fashion, partly retracing their steps and winding up at a stalk of corn within a few inches of their retreat, where they will commence to feed and continue to feed long after their companions have sought their shelters for their noontime siestas. These aimless wanderings seem decidedly more common during the morning hours than they are during the evening hours. It is for all the world as if these fellows had after a night's rest arisen refreshed in body and started out on a journey of adventure to the far recesses of their domain. At first it was supposed that these wanderings were for the purpose of seeking mates, but, so far as our observations go, bill bugs possessed with this wander-lust do not seek mates. In fact, in the majority of cases, as discussed below, coupling seems ordinarily to take place before the bill bugs emerge from their retreats.

The mating habits of the bill bugs are rather interesting. The males for the most part seek the females while they are still in their retreats. Coupling takes place usually at this time. Actual copulation does not usually take place until the bill bugs have come out from their retreats, the female carrying the male about until she has found a suitable stalk in which to oviposit. Then, while the females are making the egg cavity, copulation usually takes place. Copulation is usually completed long before the egg cavity is completed; the male, however, may continue to cling to the female for a considerable time after copulation, not infrequently clinging until oviposition is completed and sometimes clinging until the female starts another egg cavity. Sooner or later, however, the male releases his hold on the female, usually crawling away to another stalk of corn, where he commences to feed, or, if the day be well advanced, he may return directly to a retreat. This results in very promiscuous matings, for in no case have the same individuals been found mated on consecutive days.

The time spent by individuals in feeding on a given plant varies greatly. Usually bill bugs after becoming established on a plant will remain as long as an hour, frequently for several hours. Records have been made of bill bugs feeding on the same plant for at least twelve hours, but such prolonged periods are rather unusual. In all such cases the bill bugs have been protected from the hot rays of the sun by overhanging corn leaves or they have been protected by being in a group of cornstalks.

In approaching a stalk for feeding purposes, the bill bug seems to locate it merely by accident, often passing within a fraction of an inch of a stalk that is apparently as good as the one finally selected. So far as observed, a stalk had to be directly in the path of the bill bug before it was selected. Frequently observations were made which would lead one to believe that if the bill bug could not escape it would proceed to feed on a stalk of corn that directly blocked its path. No cases have been observed of a bill bug emerging from its retreat and going directly to any stalk of corn; and in very few cases was the stalk of corn nearest the retreat selected, and then only after aimless wanderings, which usually caused the bill bug to cover many times the distance required. When an adult finds its path directly blocked by a stalk of corn it pauses for a longer or shorter period of time. Eventually it crawls up the stalk for a short distance, usually from one and a half to two times the length of its body, then turns deliberately around with the center of its body as a pivot until its body is parallel with the long axis of the cornstalk, with head downward. Usually it is then half the length of its body above the surface of the ground. It then crawls down the stalk until it reaches the surface of the ground, when it may insert its snout into the stalk or it may bury itself partially or completely below the surface of the soil. In making the egg-laying punctures the jaws usually tear away portions of the outside leaves, but in making feeding punctures the bugs seem for the most part to devour the substance of the stalk as the snout is inserted deeper into the stalk.

OVIPOSITION

Age of Adults At Beginning

In the insectary during the summer of 1915 all the female beetles commenced to lay eggs within two weeks of the time of emergence from the pupal cells, if they were provided with suitable food. Inasmuch as the beetles usually linger in the pupal cells from five to seven days after they have transformed to adults, we are justified in saying that adult females commence laying from two to three weeks after they have transformed. This would mean, then, that the first adults that emerge in the early summer would commence to lay eggs that season and continue right through with their parents; whereas the beetles emerging late in the season would not find suitable corn in which to oviposit and would seek hibernating quarters and not commence egg laying until the following season. This, perhaps, accounts for the great variation in the numbers of eggs laid by individual females. (See egg-laying records above.)

EXAMINATION OF PLANT BEFORE BEGINNING OVIPOSITION

So far as our observations go, the adult female makes no preliminary examination of the plant in which she is going to oviposit. Instead, as discussed above under "Habits of the Adults," she simply seems to take any plant that offers. Neither is there any attempt to avoid plants already containing eggs. In one case, early in the season, a small plant was examined which contained five eggs, yet there were within a radius of six inches not less than four other plants that contained no eggs and many others in the same field near by which contained no eggs or only very few eggs.

The females which are making egg punctures may be readily distinguished in our experience from females that are simply feeding by the following characteristic actions. Feeding adult bill bugs either male or female take their position head downward on the stalk at or below the level of the ground, usually higher on the plant than egg-laying beetles. The body of the feeding beetles, so far as we have observed, remains motionless, the tarsi only being moved and the beak seeming to sink without effort into the stalk. On the other hand, adults preparing egg cavities seem to take a position lower on the stalk than feeding beetles, and, in addition, their bodies are pulled back and pushed forward as the beak is inserted; thus instead of a small round puncture a long oval puncture results, the beak acting somewhat as a wedge to separate the stalk as it is inserted deeper and deeper. While making the egg puncture the adults have their bodies parallel with the long axis of the stems of the plant, with head downward (Fig. 5), but when the egg cavity is finished they turn slowly about with the center of the body as a pivot and locate the egg cavity with the tip of the abdomen (Fig. 6). The tip of the abdomen is then slowly inserted into the cavity, the beetle retaining its hold upon the plant largely by means of the metathoracic and mesothoracic legs. Not infrequently the prothoracic legs are entirely withdrawn from the stalk. Oviposition completed, the beetles simply crawl away, sometimes going to shelters under clods or elsewhere, sometimes wandering about until they locate another stalk upon which they may feed or oviposit.

PLACING THE EGG

So far as our observations go the eggs are laid below the surface of the ground either in the stem of the plant or loosely among the roots. In young plants the eggs are simply placed in cavities eaten into the stem, but in older plants the eggs are carefully inserted in small pockets formed in the heavier outer leaves.

Only a few individuals have been observed to lay more than a single egg per day, but in all individuals observed laying more than one egg per day the first egg laid was deposited more carefully than later eggs. From this we have concluded that females that lay eggs loosely in the ground have deposited more than one egg for that day.

TIME REQUIRED FOR EACH INDIVIDUAL ACT OF OVIPOSITION

No hard and fast rule can be given about the length of time required in each individual act of oviposition. In general, the act may be divided into the following steps: (*a*) locating the plant, (*b*) turning head downward, (*c*) making the egg cavity, (*d*) turning with head up the plant and inserting the ovipositor, (*e*) ovipositing, (*f*) seeking shelter or another plant. Any one of these steps varies greatly with not only different beetles on different days, but with the same beetle at different times of the day and on different days. Any one of the steps may be hastened through in a few minutes or it may be prolonged for an hour or two. Sometimes one process is hastened forward with the greatest dispatch—perhaps, till it is completed; perhaps, till it is only partly completed; to be followed by a period when the beetle evidently does no work at all. If the beetle is disturbed in any way while engaged in any of the processes of ovipositing, it remains perfectly quiet for a longer or shorter period, but in no case observed did the bill bug leave the process of oviposition incompleted unless it was violently removed from the plant on which it was ovipositing.

Many beetles have been timed at all hours of the day, and, as a general rule, beetles are slower to complete the act of oviposition in the morning than they are in the evening, and slower in the evening than they are during the middle of the day. The following example of a bill bug observed almost constantly from about 6:50 a. m. until 11:45 a. m., during which time she laid two eggs, will be fairly illustrative of the time involved in the various steps of the whole process: An adult female, attended by a male, was first noted near a clod, under which there were two other females and a male, about 6:50; wandered aimlessly about until 7:05, when she approached a plant: end of step *a*. She stood perfectly still at the base of the plant until 7:18, when she started to crawl up the plant, turning around, head downward, this step (*b*) being completed a few seconds before 7:20. Worked on the egg cavity from 7:20 to about 8:05; last observed at 8:00 o'clock, not again observed until 8:06, when she had withdrawn her beak and had turned her body so that its long axis was at about an angle of 10 degrees to the long axis of the plant. At 8:09 she had completely turned around, so that her abdomen was in the egg cavity, where it remained until 8:40. When the abdomen was withdrawn the male dropped off the back of the female without having copulated, and, after rolling away from the cornstalk a distance of three or four inches, crawled away to a stalk in the next row of corn and commenced feeding. The female remained with head up the stalk until about 8:50, when she started away in the opposite direction, proceeding in the usual manner of adult bill bugs by moving rather rapidly for a few inches, then remaining motionless for a longer or shorter period of time. She arrived finally at a stalk of corn in the next row, a distance of less than five feet, at about 10 o'clock; she then remained motionless at the base of the stalk for about 35 minutes, finally turning head downward hastily at 10:35 and commenced an egg-laying puncture. This cavity was completed by 11:15, when she turned around hastily, taking a little less than a minute to insert the tip of her abdomen in the cavity, from which it was withdrawn at 11:29; within another minute she had crawled away from the stalk a distance of three inches, where she remained in the shade cast by a weed until 11:45, where further observations were abandoned for the time. At shortly after 12 o'clock the same female was found under a clod within about five inches of the place where our observations had been abandoned.

EGG AND FEEDING PUNCTURES

If there is a characteristic difference in the method of making egg cavities and feeding punctures, as all our observations would lead us to believe, then we have a ready explanation of the reason that we have two kinds of punctures in the leaves of corn plants that have been injured by the bill bugs, one being circular in outline and the other oblong (Fig. 7). We believe from observations that have been made on young, rapidly growing plants where beetles were permitted to feed only, and in other cases where they were observed to oviposit, that the circular holes represent feeding punctures and the oblong holes represent egg punctures. At one time it was thought that this would enable one to readily determine the number of eggs laid in a field, and then, by carefully counting the number of females, to be able to calculate the average number of eggs laid by females in the field; but this was abandoned when it was discovered that eggs were frequently laid loosely in the ground.

EFFECTS OF THE ADULTS ON CORN

The adults do a great deal of damage to young corn by inserting their beaks into the stem, usually below ground, and eating out the tender forming leaves (Figs. 10 and 63). This soon makes itself apparent by

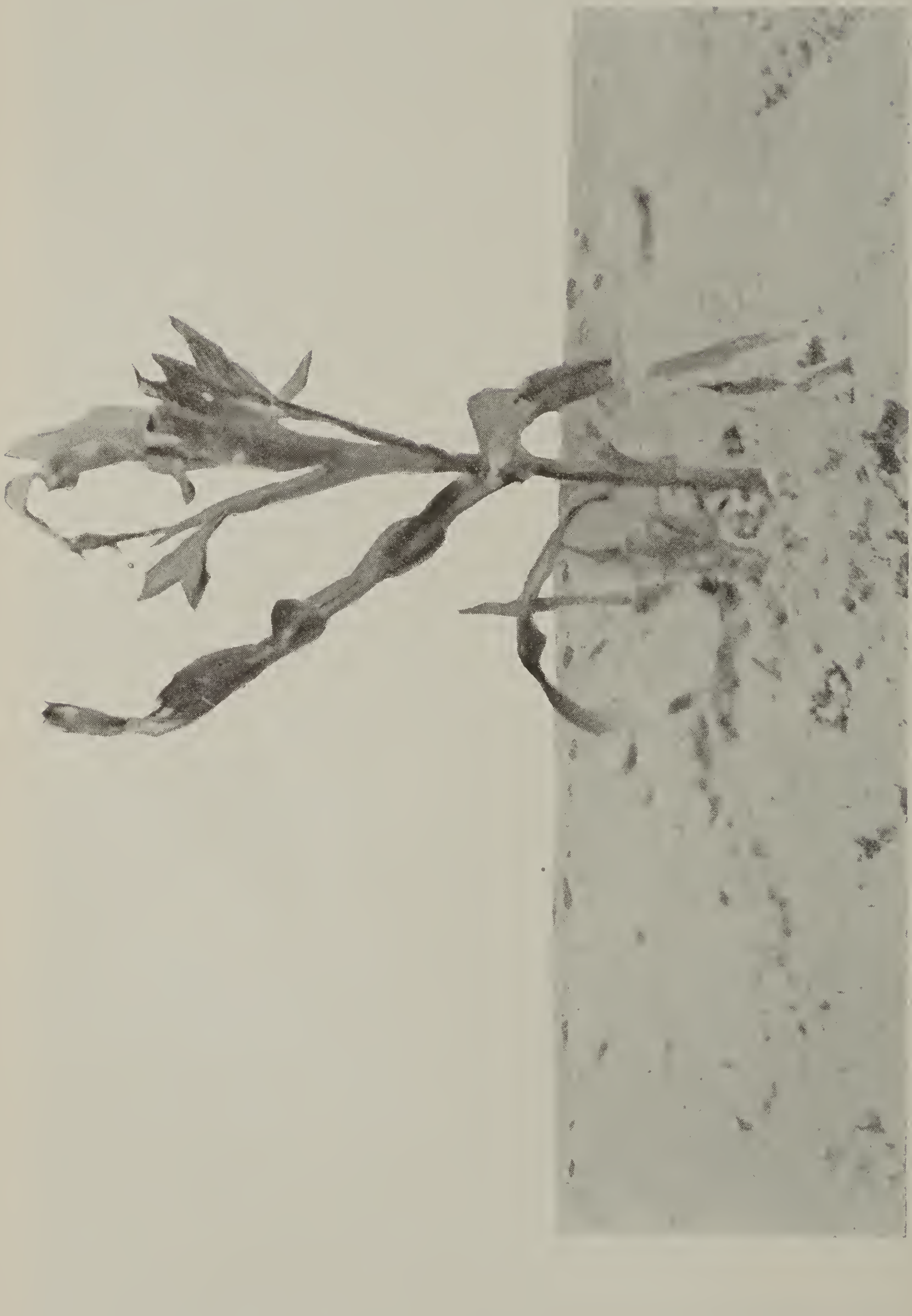


FIGURE 63. Corn stalk showing characteristic injury by adults.

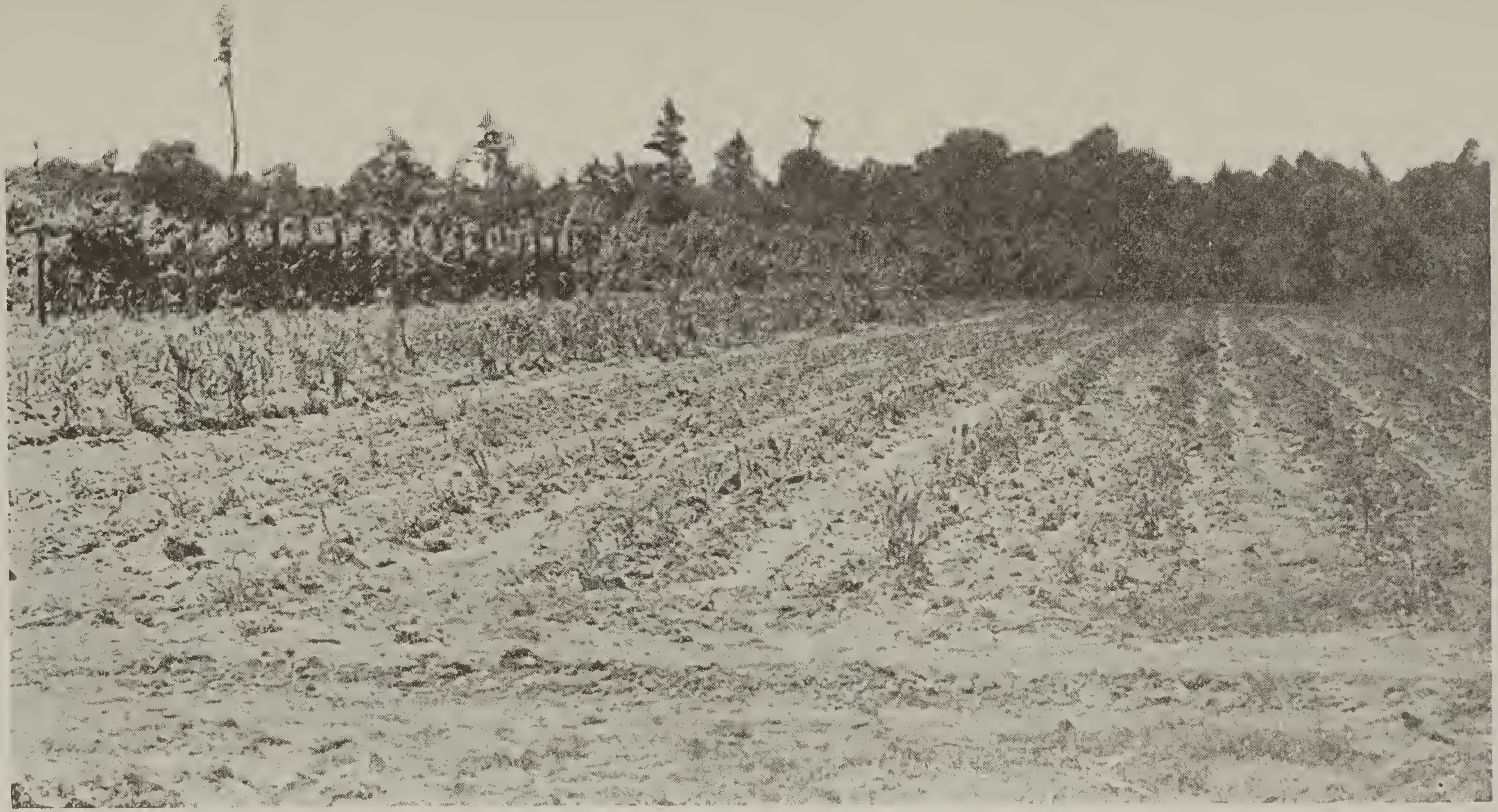


FIGURE 64. Experimental plot showing corn on the left too old to be much injured by adults. Corn on the right being badly injured by adults.

rows of round or oblong holes running across the leaves. Frequently these rows of holes are found in the so-called seed leaf, indicating that the adults often attack corn as soon as it appears above ground. When young plants are attacked in this manner they never recover, but simply die. It is hard to arrive at a satisfactory estimate of the percentage of plants that are injured in this way, for in all of our experimental plats planted to test this phase of the problem we have had to deal with bad outbreaks of the Southern Corn Root-Worm, and while the methods of attack of these two insects are entirely different, yet frequently the same plants are attacked by both insects and under such conditions it is impossible to say which insect was responsible for the death of the plant.

After the plants have grown to some extent they are seldom killed outright by the adult corn bill bugs (Fig. 64). Their growth, however, is much stunted if the adults attack it frequently; but the adults do not seem to injure corn after it has started to grow to nearly the same extent that the larvæ do.

HIBERNATION

The adult is the only stage that has been found during the hibernation period. Hibernating adults have been found in three situations: among the roots of cyperus grass, in the larval burrows in stalks of corn, and in the pupal cells in the ground among the corn roots. Many hibernating bill bugs have been found in the field during the past three winters, and of the number found fully 90 per cent have been in the pupal cells in the earth underneath the corn stubbles. A few beetles have been found among the roots of clumps of cyperus grass at Raleigh and a few have been found in cornstalks at Willard. Those that occur in pupal cells in the ground seem for the most part to be late maturing beetles, and are more common in late corn, and especially in late corn that has been retarded in its growth, as corn growing under trees and in poor soil and other like situations.

The adults in the pupal cells in the ground are apt to occur anywhere among the roots, but they seem to be especially abundant directly beneath the tap-root. The former, perhaps, represent larvæ that have developed among the roots, while the latter undoubtedly represent larvæ that have developed in the stalk; for in practically every case where there are larval burrows in the stalks of late corn the adults will be found in pupal cells near the tap-root. On the other hand, farther away from the tap-root there are usually no evidences of larval burrows in the stalk. Most of the pupal cells in the ground are situated rather deep, usually an inch or two below the bottom of the cavity caused by pulling up the stubbles. In fact, it is very rarely that one finds a hibernating beetle by pulling up the corn stubbles, unless the beetle happens to be in the larval burrow in the cornstalk. In February, 1913, I pulled up over three hundred corn stubbles in a corn-field where the corn bill bug damage had been very serious during the summer of 1912, and did not find a single corn bill bug until I took a spade and spaded the stubble out of the ground, taking care to get about three inches below the level where the roots would ordinarily break if the stubble had been pulled out directly. Then by carefully splitting the stalk lengthwise and dividing the soil below the tap-root, a bill bug could be found under practically every stalk that showed indications of a larval burrow. This occurred in about 60 per cent of the stalks in the same part of the field and in adjacent rows to those where we had pulled up over three hundred stalks and had not found a single adult. Occasionally one would find the lump of soil sometimes at a distance of five or six inches from the tap root. Perhaps more beetles occurred in such situations than we actually observed, for in the winter, when bill bugs are inactive, they are exceedingly difficult to distinguish from lumps of earth. When one was found in such situations it was usually due to the fact that the lump of earth adhering to the roots happened to break across the pupal cell, which would usually be conspicuous because it was a cavity with a smooth surface. Discovery of the pupal cell usually led to the discovery of the beetle; sometimes it was found projecting from the other half of the cell, but more often it was found among other little lumps of earth, from which it could usually only be distinguished by individual examination.

Thus it seems safe to conclude thta the beetle stage is the only one that ordinarily passes the winter, for many adults have been discovered in hibernation and no other stages have been found. The only apparent exception to this statement is the record of a single pupa found by Mr. Hyslop, as recorded below; but, as explained elsewhere, the larvæ that develop from the last eggs laid in corn undoubtedly do not reach maturity before early November; hence this record would have to be excluded as a hibernating individual. Of the individuals that have been found in hibernating quarters, about 90 per cent were in pupal cells in the ground below the tap-root and about 2 per cent in the larval burrows in the stalks, and a little more than 7 per cent were in pupal cells among the roots.

The peculiar situation of the pupal cells perhaps accounts for the fact that others have had difficulty in locating hibernating bill bugs, as Mr. Smith's record will show.

"The bill bugs hibernate ordinarily in the beetle stage. This statement is made without reservation and is based on many field observations during the winter of 1909-1910, when careful search on several occasions failed to reveal any eggs, larvæ, or pupæ in the field where bill bugs were known to have bred during the previous falls. It has proved to be extremely difficult to find any bill bugs in their winter quarters, but the total absence of larvæ and pupæ in the breeding places proves that the winter is not passed in either of those stages.

"Only one record has been secured of actually finding the beetles in a place where they presumably pass the winter. On November 4 and 5, 1911, in company with Mr. J. A. Hyslop of the National Bureau of Entomology, adult bill bugs were found in corn stubble in a field on Harvey's Neck in Perquimans County. The trip to this field was made for the express purpose of trying to find the beetles, for it was assumed if any were found on that date that they would be in hibernation for the winter. After considerable search we located a dead beetle in a cornstalk. This beetle was at a point in the stalk about even with the surface of the ground. Soon afterwards we found another dead beetle in a similar location. On the second day three live beetles were found in cornstalks in a field where serious bill bug damage had occurred during the summer. Apparently these beetles had matured late and had not attempted to escape. We consider this to be proof of the hibernation actually occurring in the cornstalks. The beetles were in the stalk near the roots where the larva had fed and transformed.

"During these two days when beetles were found in cornstalks, we also searched for the beetles and the immature stages, in cyperus plants, but without success. In fact, there were only a few cyperus plants found in which Sphenophorus larvæ had lived. The writer believes, however, that the bill bugs had bred in cyperus in these fields in plants that had died down and become invisible.

"Hibernation must also occur in the soil, or under the protection of some material in the fields, as well as in cornstalks. The breeding of beetles during the past summer, when every reared specimen emerged and fed nearly every day thereafter until the middle of November, shows that it is the natural thing for the beetles to come out and feed after maturity. If this is true, the majority of the bill bugs are not hibernating in cornstalks unless they crawl into the stalk when cold weather commences. That this actually occurs is extremely doubtful. The beetles found in the cornstalks had certainly never emerged, and no beetles were found under conditions to indicate that they had crawled into the stalk.

"On November, 5, 1911, Mr. Hyslop found one healthy live pupa in a cornstalk. This indicates a possibility of the pupæ living in that situation all winter. The rearing records, however, do not indicate that pupæ are liable to live all winter. This point needs further investigation, but in view of present knowledge we may consider that only the mature beetles normally live during the winter.

"The writer has searched for the beetles in winter quarters in fields at Lumberton, Chadbourn, Braswell, Pembroke, and other places, including the fields around West Raleigh, where many bill bugs bred during the last two seasons. Without recording details of the work, it may be stated that the beetles have never been discovered during the winter months, that is, December to March, inclusive, except in one instance, at Chadbourn, when a single specimen was taken in the edge of a rice field on March 29, 1911. On that date one beetle was found in a clump of grass, where it was moving about; hence it was not exactly in hibernation. Sticks, stones, logs, dead grass and weeds, brush, and, in fact, every movable thing in the field, have been overturned in the search for beetles in hibernating quarters other than cornstalks."

Just what becomes of the early maturing adults has never been determined. During mid-season the adults seem to emerge from the pupal cells in from five to seven days after transformation; later in the season this time seems to be gradually extended, and the late beetles remain in the pupal cells all winter. The writer believes that the early maturing beetles leave the cornfield late in the season, especially if the corn has been planted early, and migrate to later corn or to adjacent patches of cyperus grass, where they undoubtedly go into hibernation anywhere in the field a few inches below the surface. No beetles have been found save those that were still in pupal cells, in spite of the fact that we have sifted the top five or six inches of soil in many different parts of various cornfields that had been badly infested with bill bugs the previous season. Any one, however, who has had any experience with bill bugs in the field will appreciate the difficulties involved in attempting to locate bill bugs by sifting, and it is not at all remarkable that we were unable to locate them.

BEETLES FROM EMERGENCE TO HIBERNATION

Just where the early maturing adults spend their time from the time when they emerge from the pupal cells till they go into hibernation is still in part an unsolved problem. Part of the adults, evidently late maturing ones, remain in the pupal cells over winter; and part of the early maturing adults emerge from their pupal cells and feed in the same fields with their parents. Whether these young beetles lay any eggs the first season or not is discussed in another connection. In fields of early or medium early planted corn the corn reached maturity in early September, and in such fields all the adult bill bugs have practically disappeared by late August. In later planted corn, especially in extremely late corn, the stalks may stay green and tender until after heavy frost, and the adults continue in such fields until late October, at least, evidently not seeking hibernation quarters until early November. In most cases, however, no such late corn is available, and it therefore becomes a problem to determine just where the adult bill bugs live from late August to early November. In the hope that we would be able to solve this problem, something in the neighborhood of five hundred pairs of adult corn bill bugs were marked in various ways in early August, 1912.

The adults marked in 1912 had a spot of bright water color painted either on the thorax or the elytra, as this method of marking had been previously tried out very successfully in studying the diurnal activities. However, none of these beetles were ever seen after the last of August. Believing that the water-color paint used during 1912 might have been removed in the ordinary daily activities of the beetles, other pairs were marked by clipping their elytra during August, 1913 and 1914. None of these beetles were seen after mid-September and only a few after late August. Not many were ever seen far from the place where they had been marked, although a few were recovered in adjacent fields. None of the beetles marked in 1913 were recovered in 1914, and none of those marked in 1914 were seen in the spring of 1915.

However, in spite of this negative evidence, the writer is of the opinion that the beetles leave the early corn-fields in late August, either traveling to late fields of corn or to patches of cyperus grasses. This conclusion is strengthened by the fact that the bill bugs seem to disappear from the corn-fields very suddenly, and there is some very strong evidence from observations made in the insectary to show that late in the season the adults seem to develop a sudden instinct to fly. This is perhaps true in the field, also, and would naturally account for the sudden disappearance of the beetles from the corn-fields, as in the insectary all the beetles seem to acquire the instinct to fly at about the same time.

We have made no direct observations in the field that the beetles fly, save the indirect deductions as outlined above. If the beetles do acquire the instinct to fly from the fields, it would aid very materially in dispersing the species and would be a very important phase in their life cycle.

Careful counts of the numbers of beetles per acre have been made on small plats averaging one-third acre each. In these counts an effort was made to secure every beetle present in the field. Some were undoubtedly missed, but our counts usually showed an average of about 1,100 beetles per acre in mid-August, this number dropping so suddenly that by the last week in August there would be on the average from 75 to 100 beetles present per acre.

MORTALITY DURING HIBERNATION

The mortality during hibernation has varied greatly in our insectary experiments. It has ranged from about 10 per cent in some cages to over 90 per cent in other cages. Beetles for our hibernation experiments were placed in various kinds of cages. Some were placed in jelly glasses with varying amounts of sand and varying numbers of beetles in each glass. The mortality seemed to increase with the increased numbers of beetles in a single jelly glass. Some beetles have been held over winter in large screened cages, where they had on the average about six inches of soil in which to bury themselves. These cages were exposed out of doors, and the beetles usually went into the soil to a depth of from four to five inches. In those cages that were left undisturbed the mortality was usually only about 10 per cent. A single disturbance of the beetles seemed to increase the mortality very much, and in cages where the beetles were disturbed several times they all died.

In the fields careful counts of plats averaging about one-third acre show that normally the numbers decrease from a rate of about 1,250 per acre in July of one season to about 420 per acre by the end of May the following season, by which time it is believed all of the adults have emerged from hibernation. This would give a mortality of about 66 per cent, provided all the beetles returned to the same fields; but, as pointed out in another connection, we have not been able to prove that any of the beetles returned to the same fields in which they worked the previous season, although, in spite of our negative evidence, some beetles undoubtedly do return to the same fields.

NATURAL ENEMIES

The corn bill bug is singularly devoid of natural enemies in all its stages. So far in these investigations no parasitic enemies of any of the stages of the bill bug have been discovered, and only a very few predaceous enemies. In the corn-fields in the eastern part of the State adults and larvæ or various species of ground beetles, lampyrid beetles, digger wasps, robber flies, and other predaceous insects, abound. Various species and individuals of these predaceous insects have been watched often for considerable periods. There is no indication that these insects prey upon any of the stages of the corn bill bug. Even when eggs, larvæ, and pupæ were exposed directly in the path of these predaceous insects they made no effort to devour them.

The little brown corn-field ant is also constantly present in the fields, and these have frequently been observed with eggs of the corn bill bug in their jaws, evidently having picked up eggs that were carelessly deposited or that had been deposited in the ground among the roots. There is no evidence that this ant enters the egg cavities and removes eggs that have been carefully deposited, although they are constantly running up and down the stems of corn plants, especially corn plants infested with root lice. In fact, the eggs are for the most part so well placed that it is rather doubtful whether ants would be able to secure any carefully placed eggs. However, ants have been seen with eggs so frequently in the field that one is led to suspect that they destroy a great many eggs in this way. Mr. Smith well says (Smith, 1913): "An egg dropped or placed on the ground or exposed on the plants is very quickly taken away by the little field ants. Just how much benefit they cause by destroying the eggs the writer has not had a chance to discover."

In October, 1915, I chanced upon the following observation, which is given in some detail because insect enemies of the corn bill bug are so rare. In pulling up a stalk of corn in a field which had been planted in early July, I discovered a larval Tabanid firmly anchored to a larva of a corn bill bug of large size which was evidently nearly ready to pupate. It took considerable force to dislodge the Tabanid larva, which was placed in a tight tin box with four other good-sized corn bill bug larvæ and two pupæ. After a few minutes the box was examined and the Tabanid larva had again attached itself by its mouthparts to one of the corn bill bug larva. The box was placed together with some other boxes and brought back to the laboratory, where opportunity was not offered for an examination until the following day. By this time the Tabanid larva had died, although it had completely eaten the four larvæ and one of the pupæ with the exception of the hard outer chiten and had eaten a hole into the side of the other pupa.

On the face of this observation it would look as if Tabanid larvæ might be very important enemies of Sphenophorus larvæ and pupæ, especially those that are exposed in the ground among the roots of the corn plant, for the predaceous habits of Tabanids are well known and they both occupy the same sort of situations generally, *i. e.,* moist, swampy situations, and must, therefore, be thrown constantly in contact. However, this single observation is the only evidence we have bearing on this point, and we have examined hundreds of larvæ and pupæ in the fields both in cyperus swamps where the natural conditions for the Tabanid larvæ would be good and in corn-fields where the natural conditions would not be so good. The same day the above observations were made we spent much time searching for other Tabanid larvæ, but none were found either in the field where corn bill bug larvæ abounded or along the ditch banks under very favorable conditions for Tabanid larvæ.

Mr. Smith was much troubled in his investigations by a small mite which invaded the insectary. Of these mites he makes the following statement (Smith, 1913):

"Mites have proved to be a pest of both the larvæ and the pupæ in confinement; but field observations do not show that the same thing is true under perfectly normal conditions. During the breeding work many larvæ are apparently killed, or worried so as to prevent regular growth, by a small mite identified by Mr. Banks as *Tyroglyphus americanus* Bks. These mites were often found on the bodies, or in their feeding channels, and usually such larvæ did not grow well. Very young larvæ were sometimes killed by the mites."

During 1913 the insectary was moved and we have had no further trouble with this mite. Neither has the mite ever been observed in the field in the natural burrows.

A good deal of trouble has been experienced in our breeding work with both larvæ and pupæ, which had apparently died from attack of some fungous growth. However, such larvæ were usually found in stalks of corn that were badly decayed or molded, and the chances are that the larvæ had died from the want of proper food and the fungus had only attacked the larvæ or the pupæ secondarily. Concerning this point, Webster (1912) quotes Dr. Chittenden as follows:

"In another instance, during the last week of August, larvæ of this same species were dying and specimens were referred to Dr. Haven Metcalf, a Pathologist in the Bureau of Plant Industry, who stated that they were apparently free from fungi, and that while there was a possibility of the presence of a bacterial disease, such presence could not be established at that stage. Examination, however, revealed the fact that the bodies of the larvæ were fairly reeking with nematodes, and it is not impossible that these are the cause of the insect fatality."

In our observations no nematodes have been found either in the insectary or in the field. A condition similar to the above seems to be very prevalent among young larvæ, especially late in the season, and for this reason we have concluded that the death of the larvæ was due to poor food.

Toads also abound in corn-fields infested with corn bill bugs, but there are no direct observations to show that toads eat corn bill bugs in any stage. Neither has the examination of toads' stomachs revealed the presence of bill bugs. As a matter of fact, from direct observations one would almost conclude that the toad's eyesight was not good enough to distinguish the slow moving bill bug, whose natural color is so similar to the soil in which it lives. Attempts to feed bill bugs to toads have always resulted in the toad ignoring the bill bug.

Only one bird other than the domesticated fowls has been recorded as feeding on corn bill bugs. According to Webster (1912), "Mr. W. L. McAtee of the Biological Survey has recorded the findings of *Sphenophorus callosus* in the stomach of the nighthawk (*Chlordeiles acutipennis texensis*) at Wallaceville, Texas, August 4, 1907. This is the only exact record obtainable of the eating of this species by birds."

Nighthawks are very common in the eastern part of this State, especially late in the season. But their well known habits of catching their prey on the wing has earned for them the distinctive name, "Bull Bat." This habit would seem to preclude these birds from being considered as important enemies of the bill bugs, unless, as pointed out in another connection, the bill bug has a fall dispersal flight, which would naturally come at about the same time as the greatest abundance of nighthawks, due to their southern migration.

Chickens and turkeys are believed by many farmers to be important enemies of bill bugs, and this may well be true, but we have made no direct observations on this point. One farmer in the eastern part of the State says that several years ago he had to yard his chickens to prevent them from running in a corn-field badly infested with corn bill bugs, for the bill bugs frequently lodged in the throat of the chickens or apparently attached themselves to the tongues with their sharp mandibles in such a way as to choke the chickens.

CONTROL

The control of the corn bill bug is not an especially easy farm practice. This is due to a number of different factors. In the first place, the insect cannot be attacked by any direct remedies, both because of its hardiness and because most of its life is spent in the stalk of corn or buried beneath the ground. The only direct method that might be used to any avail against this insect is hand picking of the adults in the early spring and destroying them by throwing them into boiling water or kerosene. This practice would be too expensive to use on general crop corn, but might be used in small fields of corn which were being grown for roasting-ear purposes. If this method were to be used it would be advisable to scatter chips about the field under which the bugs would crawl during the middle of the day and from which they could be collected readily.

In the main the farmer in the bill bug section of the State will have to depend upon a slight modification of his usual farm practice to control the corn bill bug.

Careful observations made during the past several seasons show quite clearly that the control is not a very easy matter. It has also been clearly demonstrated that no one method can be used with any great success against this insect, but rather that the control is dependent upon a number of factors which, taken together, give us a system of corn farming somewhat different from the systems practiced in the corn bill bug section at the present time. These factors are discussed separately in some detail below, but it must be emphasized again that the farmer cannot put his dependence in any one method alone, but, instead, that he must work out a system of corn farming with these various factors in mind. In this connection it must also be emphasized here that a system which is built upon a basis of the factors here given is not necessarily a good system for growing corn in all sections of the State under all conditions, but rather that this is the system the farmer should employ in the corn bill bug sections of the State if he hopes to grow corn with the minimum of loss by corn bill bugs. Neither is it intended to imply that this system will avoid the attacks of other insect enemies of corn, but is simply to be used where the corn bill bug is the most important insect enemy of corn. The writer believes that the farmer who follows this system will also be doing the most important things in the control of many of the more important insect enemies of corn; but there are so many factors involved both in the many varieties of insect pests and climatic and other conditions which have not been studied in detail that it would not be surprising if certain insects were not controlled by following the suggestions as outlined here.

The writer believes that the following are the most important factors involved in any system for the control of the corn bill bug, both from the standpoint of ease and cheapness of application: (1) time of planting, (2) rotation of crops, (3) fertilization, (4) drainage, (5) ridging, (6) fall and winter plowing, (7) thorough cultivation, (8) destruction of native food plants. It will be noted at once that all of these factors are what might be called indirect methods of control. From the very nature of the case it would be impossible to apply any direct methods, for all such methods are expensive and the returns from corn are never great enough to justify the expense involved in their application.

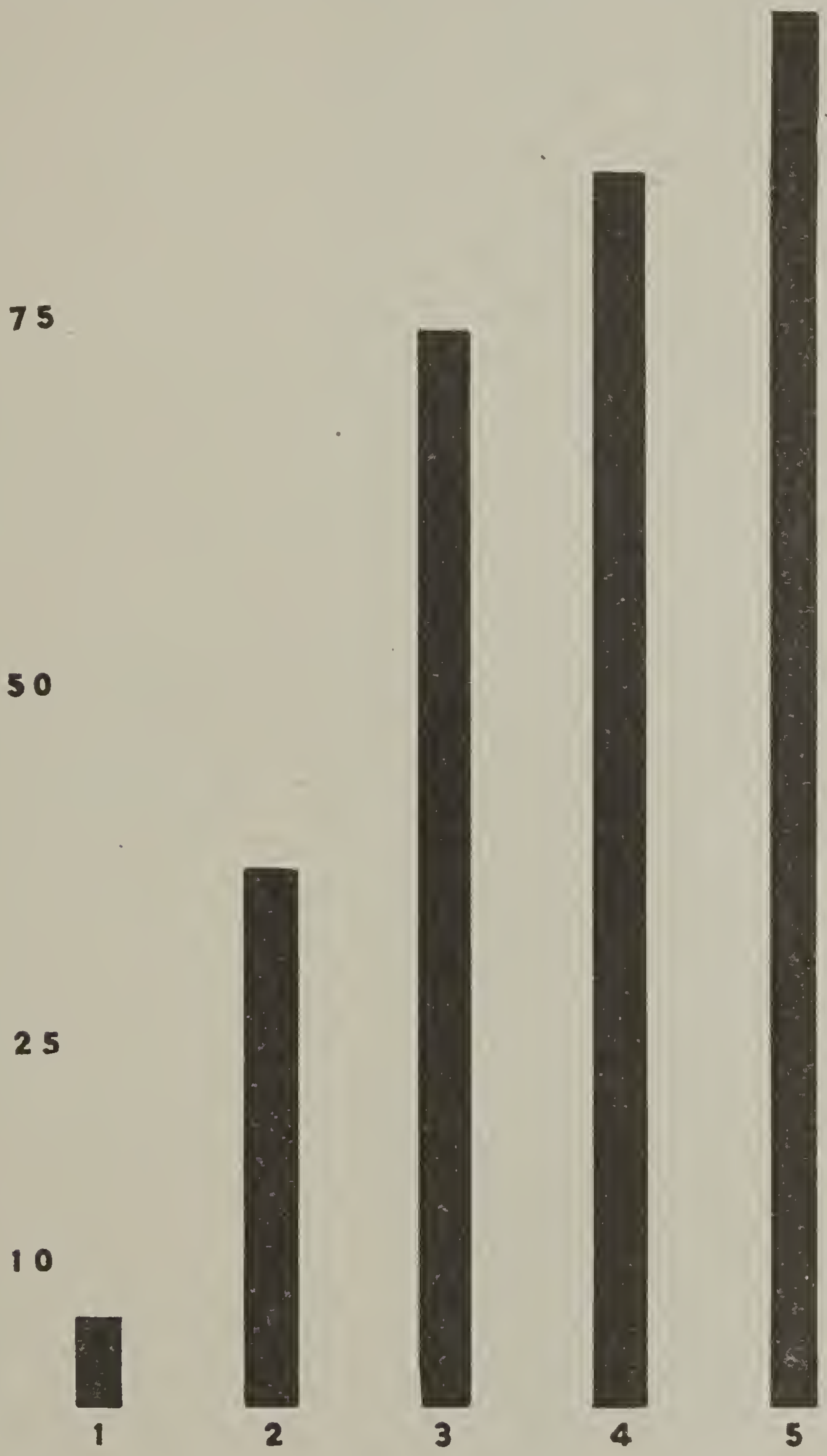


Figure 65. Diagram showing the percentage of corn injured when planted at different dates, in Pender County. 1. Corn planted late in March. 2. Corn planted mid April. 3. Corn planted early May. 4. Corn planted early June. 5. Corn planted early July.

TIME OF PLANTING

A series of experiments carried on during 1912 proved very conclusively that time of planting is an important factor in the control of the corn bill bug. Plats of corn were planted on the following dates: March 23, April 13, May 3, June 3, July 3. Careful inspections were made of these plats from time to time, and the following rough percentages will give an indication of the amount of injury:

Date Corn was Planted.	*Percentage Injured.*
March 23	6
April 13	37
May 3	74
June 3	85
July 3	94

From these figures it seems safe to conclude that in order to grow corn successfully in the corn bill bug section of the State it is necessary to plant the corn before the middle of April. However, too much dependence must not be placed in this one factor; for if all the other elements that go to make up the successful method of control of this insect are neglected, early planting alone will not insure a successful crop.

The reason that early planting is successful against this insect is undoubtedly because the early corn gets a start and is thus able to keep ahead of the attacks of the bugs. In the fields it always seems that when corn gets to be about waist high it is no longer much troubled by corn bill bugs, but that it is able to continue growing in spite of their attacks. Corn planted in late March in the eastern part of the State in the usual season has made such a satisfactory growth before the bill bugs come out of winter quarters that it continues to grow right along in spite of their attacks. On the other hand, corn planted after mid-April is just coming up at the time of the greatest prevalence and greatest activity of the adults. Fields planted at such times are so badly damaged that they cannot produce a satisfactory crop.

ROTATION OF CROPS

Rotation of crops is an important factor in the control of the corn bill bug, just as it is in the control of other important insect enemies of farm crops. This is due apparently to two reasons: (1) To the fact that if crops are moved from field to field the insects are forced to move also, because, as a general thing, insects which feed on corn do not feed upon other farm crops. In forcing the insects to move in this way, the chances are that they will not be able to find the new location, and as a result will die for the want of feed. (2) When corn is grown continuously on a field for a number of years the food which the corn requires

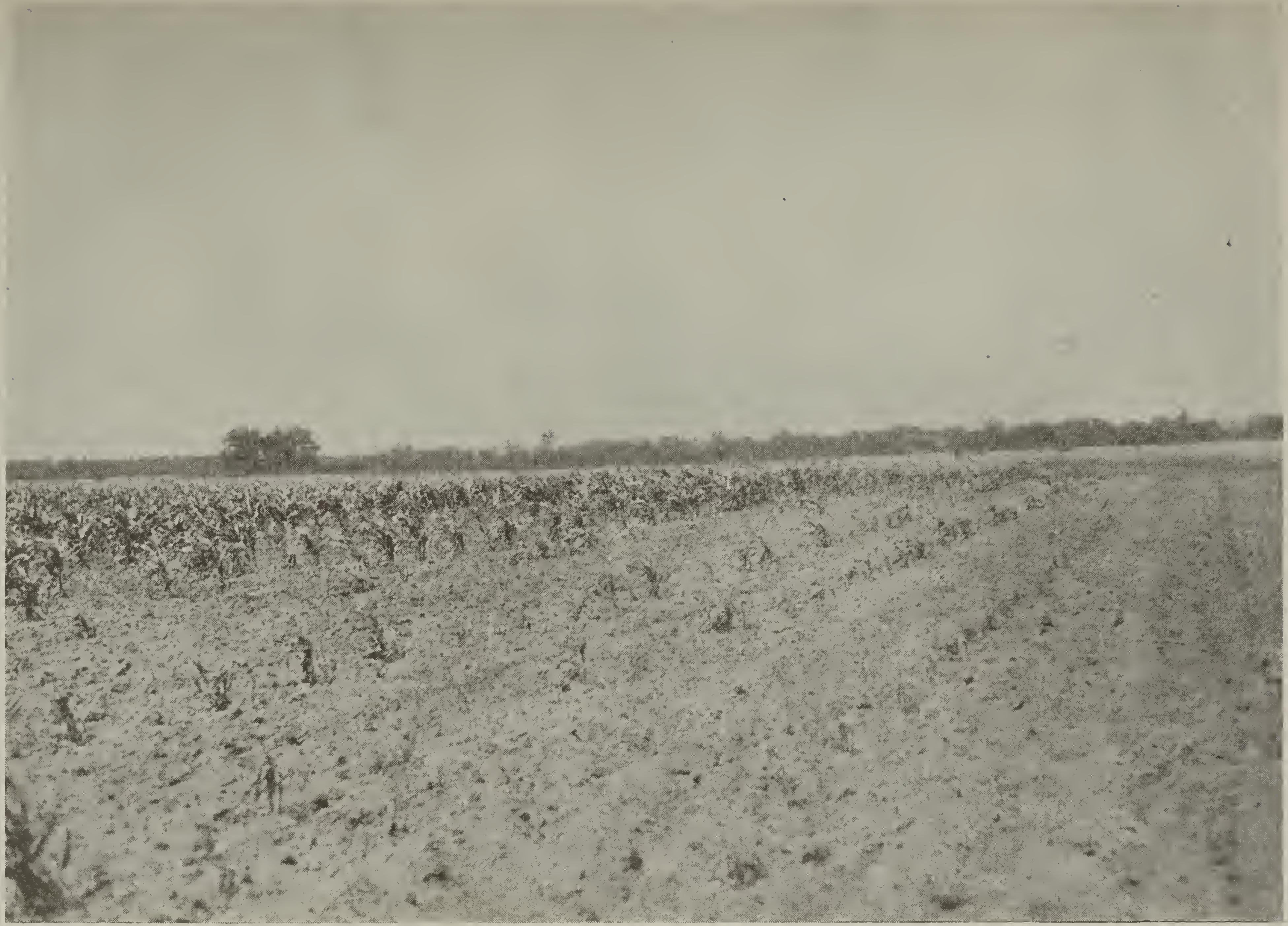


FIGURE 66. Experimental plots on Pender Test Farm showing the advantage of early planting. Corn on extreme left planted late March only very slightly injured. Mid April planting not much injured, but more injured than the late March planting. Next rows to the right planted early May very severely injured. The two rows on the extreme right planted mid May almost completely destroyed.

becomes so exhausted that the corn does not make a satisfactory growth, and hence it readily succumbs to the attacks of insects. Whereas, if proper crop rotation has been practiced, the corn would get a satisfactory start and would be strong and vigorous enough to withstand the attacks of insects to a certain extent at least.

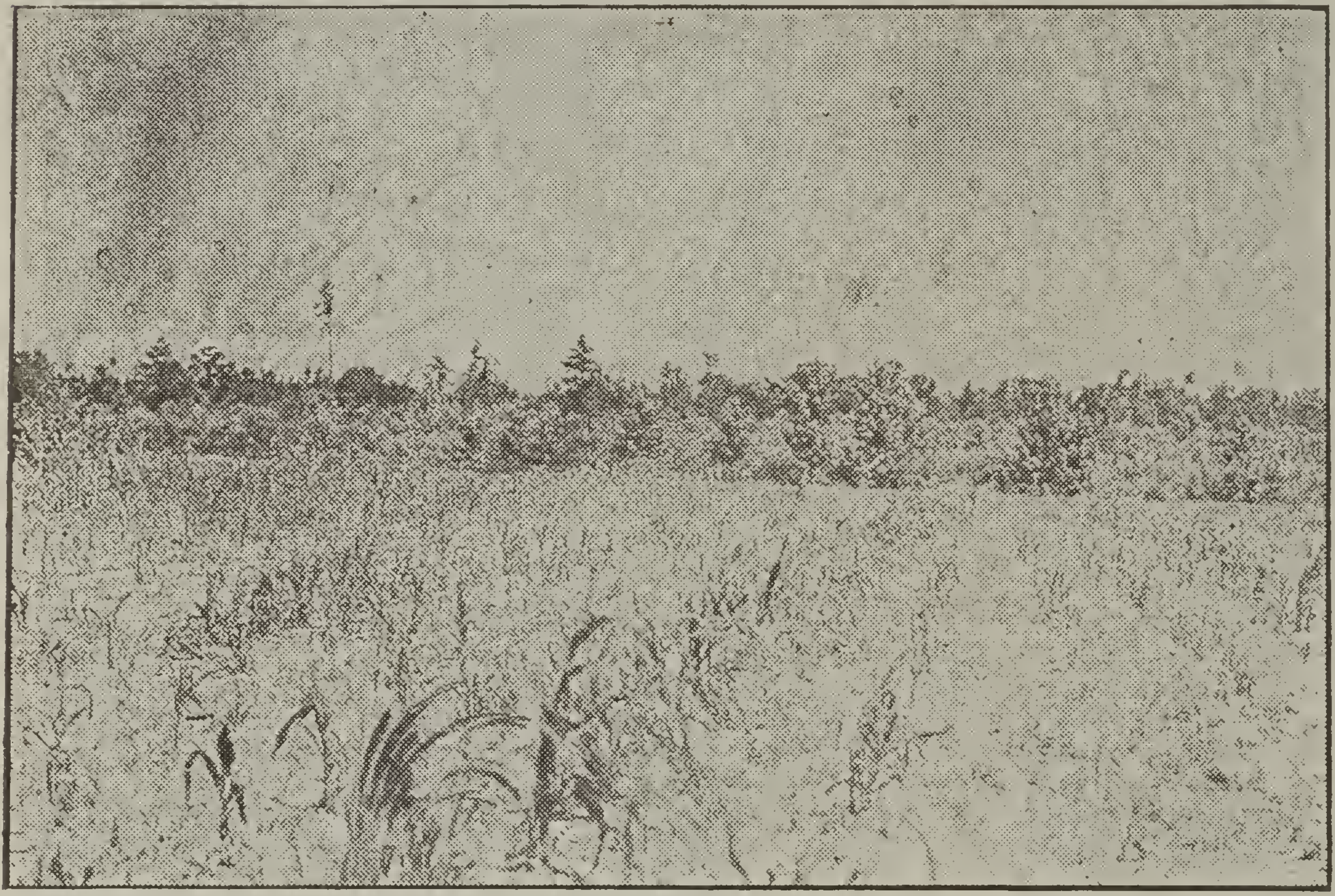


FIGURE 67. Experimental plots showing the effects of heavy applications of complete fertilizers. Row in center and on either edge of plot heavily fertilized. Other rows without fertilization.

FERTILIZATION

Fertilization is, perhaps, next to "time of planting," the most important factor in the control of this insect. This is not, of course, the proper place to enter into a discussion of the proper fertilizer to use for corn, but it has been shown on one of our experimental plats that an application of fertilizer will make the plants grow much more rapidly, and hence be less liable to be fatally injured by the corn bill bug than plants which have not been heavily fertilized (Fig. 67). In the same way a heavy application of lime to land that is sour will make the plants grow much more rapidly and thus escape the attacks of the bill bug. The importance of lime may be further emphasized by the fact that very much of our corn land in the corn bill bug sections of the State is naturally very acid.

The belief so prevalent in the corn bill bug sections that fertilizers act as repellents against the corn bill bug does not seem to be based on facts. Repeated experiments with various mixtures of fertilizers, air-slaked lime, tobacco dust, tobacco extract, and basic slag failed to show that the adult beetle was deterred from feeding and laying eggs in

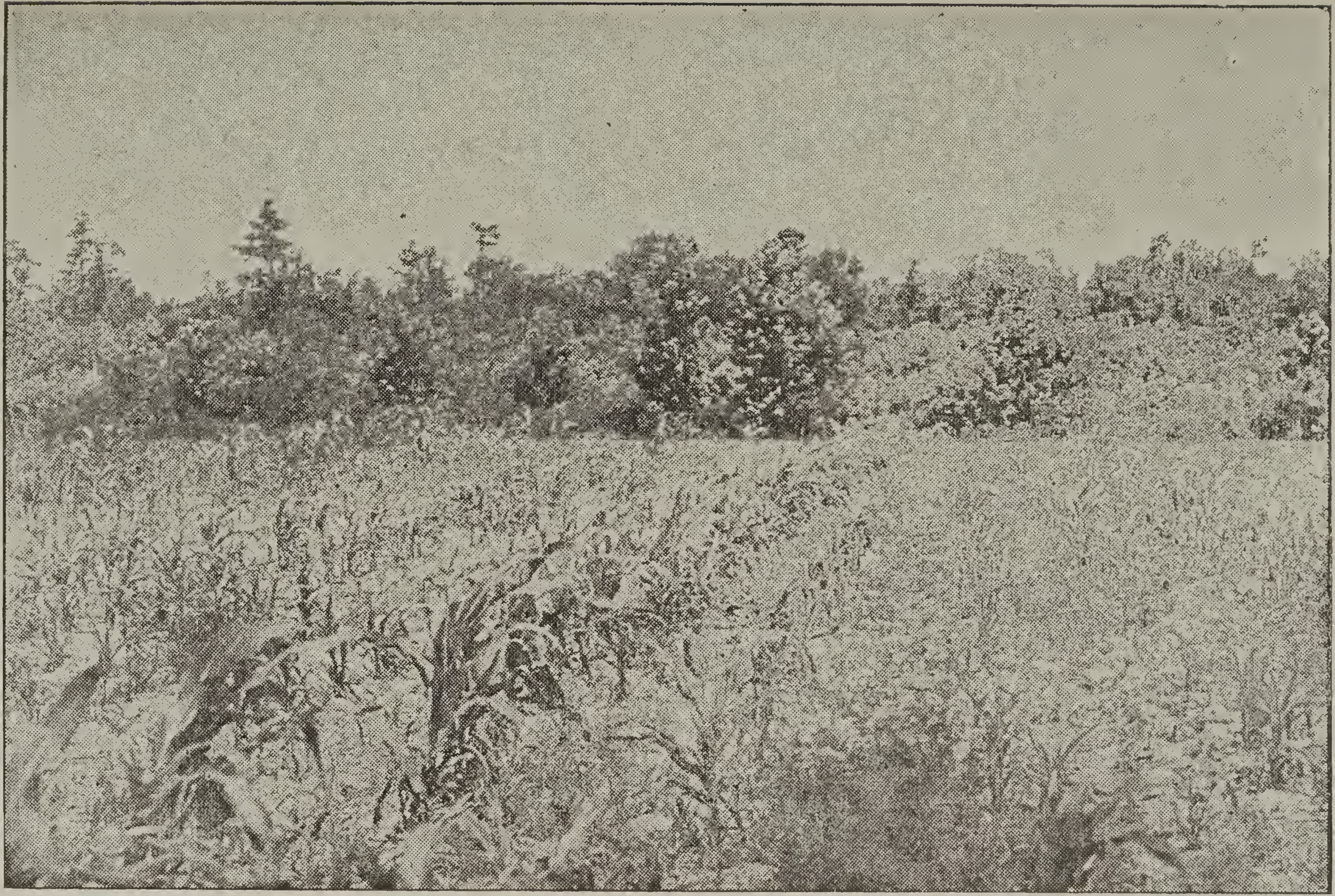


FIGURE 68. Experimental plots to show effect of ridging; row in center and either edge of plot ridged; other rows planted on the level.

plants thus protected. In some cases the material used as a repellent was actually mounded around the base of separate cornstalks, yet the beetles crawled over the mounds of the supposed repellent to feed upon the protected stalk. In all cases where a repellent action has been ascribed to any fertilizer, the writer is of the opinion that the results observed are not due to the repellent action of the fertilizer, but to the fact that such plants are growing rapidly, owing to an excess of food material; hence they escape the attacks of this insect.

DRAINAGE

It is a matter of common observation that the corn bill bug is worse in low wet lands than it is in uplands. Naturally this has led to the statement that the most important factor in the control of the corn bill bug is proper drainage of the land; and while this is an important remedy, its importance might easily be over-emphasized. The Pender Test Farm is exceptionally well tile drained, yet the bill bug has not become a negligible factor on this farm. And while we must not leave the impression that proper drainage is not an important factor in the control of this insect, yet we must caution the farmer against assuming that drainage is all important. It would not be possible for the corn bill bug to be worse than we have observed it on some well drained fields. (Fig. 2.)

RIDGING

Observations made in fields in various parts of the bill bug sections of this State show that stalks of corn which are on a slight elevation above their neighbors nearly always escape the attacks of the corn bill bugs for a longer period. Sometimes stalks which are on very slight elevations will not be attacked, while all the rest of the corn in the field has been very severely injured. The amount of immunity to attack seems to be out of all proportion to the height of the ridge. This was especially noticeable in a field on the Pender Test Farm, where many stumps had been recently pulled. In pulling the stumps a ridge of earth was left around a hole which was usually quite shallow after the plow had passed over it. Corn on this ridge either was immune from attack or was attacked only after all the other stalks in the vicinity had been seriously injured or killed. This led to the belief that if corn was planted on a ridge it would to a certain extent escape from the attacks of the insect.

This method was tried, and three rows through one of the plats (Fig. 68) were planted on a ridge which averaged about eight inches high. Corn was planted on these ridges and in rows between the ridges on June 20th. Careful examination made a month later showed that 45 per cent of the corn on the ridges had been injured, whereas 98 per cent of the corn in the rows between the ridges had been injured. Another examination showed that the corn on the ridges had been able to continue its growth and was much more advanced than the corn on the level.

This method is not generally applicable, owing to the cost of constructing ridges. It is mentioned in this connection because it might be used for the growing of early corn for market purposes where the expense involved would not be such an important item. This method must not be confused with the general practice of ridging corn at the time of the last cultivation. In the ridging method as here outlined the corn is planted upon a ridge. This method would be applicable only to lands that are very moist and where bill bugs normally abound, and not to lands that are very dry.

FALL AND WINTER PLOWING

Fall and winter plowings have been tried to a limited extent upon small plats, but no very striking difference could be noted in favor of this practice. However, if most of the adults live over the winter in the fields, the plowing of the land in the late fall or in the winter would disturb them very seriously and would act as a decided check upon their ravages. If, on the other hand, only a small number of beetles live over winter in the corn-fields, then the fall or winter plowing would not be such an important factor in the control of the bill bugs. The writer believes, however, that even if it should be proven that only a fraction of the adults hibernate in the corn-fields, the practice of fall or winter plowing would be profitable as a factor in the control of the corn bill bug where the same field was to be planted to corn two years in succession.

THOROUGH CULTIVATION

It has been stated that frequent shallow cultivation will keep the corn bill bug in control. And there are many things that would lead one to believe that this might be true. As discussed above, the adults spend considerable time hiding away under clods, etc. Therefore, it would seem but logical that cultivation would have a tendency to break up these retreats, and, if the cultivations were frequently made, that they would have a tendency to keep the insects disturbed; and these factors perhaps have some importance. However, a rather long series of observations has led the writer to believe that the effects of cultivation are not upon the bill bugs directly, but are rather upon the corn, causing it to grow more rapidly, and hence to be much more resistant to the attacks of all its pests. Observations show that corn that is well cultivated will generally make a better growth when atacked by corn bill bugs than corn which is improperly cultivated; but, on the other hand, the writer has frequently seen corn succumb to the attacks of this insect when, so far as he could determine, the corn had had the very best of cultural attention. Then, again, counts of the number of bill bugs per acre made in widely varying localities and ranging from fields in a high state of cultivation through innumerable graduations to fields that had just been cleared and never plowed, but in which the corn had been "stuck," failed to show any constant correlation between the state of cultivation and the number of bill bugs present per acre. During 1915 the corn in the experimental plats on the Pender Test Farm was given very poor cultivation and was badly damaged by corn bill bugs, but the adjacent plats which were devoted to other experiments were given very careful cultivation. Yet, so far as could be determined, both plats were about equally injured. At least, the plats devoted to the other experiments had to be replanted, owing to the great amount of injury by corn bill bugs.

However, the writer does not mean to imply by any of the above statements that corn could be grown as well without cultivation as with proper cultivation. Nothing could be farther from the truth. Yet it does seem worth while to correct a growing misconception to the effect that proper cultivation is an early effective method of control for the corn bill bug.

DESTRUCTION OF NATIVE FOOD PLANTS

The corn bill bug is undoubtedly a native insect, and, like many other insect pests, fed formerly on various kinds of weeds. So far as has been determined, these weeds were mostly grass-like plants, especially the grasses which are closely related to nut grass and chufas. The corn bill bug still continues to feed and breed on these grasses throughout its range in this State. Therefore, corn grown in close proximity to such grasses or in fields where such grasses have been allowed to grow, is more apt to be injured by this insect than corn grown in fields that are free from these grasses.

Therefore, anything that can be done to keep down the native food plants of this insect will be important factors in its control. Many of these grasses are swamp-loving forms. Therefore, as the swampy areas on the farm are brought under better and better drainage these plants will be restricted in area and will therefore furnish less food for the corn bill bug. This will lessen the danger of the insect spreading from its native food plants to corn. In this connection, the farmers in the bill bug sections of the State should pay particular attention to drainage of swampy areas where the native food plants abound, and should also watch, closely, the edges of ditch banks and similar situations to see that such places do not grow up in these grasses which would furnish these pests with an abundance of food and would act as incubators for their increase.

Also, it seems hardly necessary to say that if some other crop would be grown on swamp land for the first year or two after it is cleared, it would have a tendency to reduce the damage done by this insect.

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NORTH CAROLINA STATE COLLEGE OF
AGRICULTURE AND ENGINEERING

RALEIGH AND WEST RALEIGH

DIVISION OF ANIMAL INDUSTRY

REPAIR OF BONE IN DOMESTIC FOWL

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REPAIR OF BONE IN THE DOMESTIC FOWL

B. F. Kaupp, Poultry Investigator and Pathologist, Animal Industry Division

In presenting this material the work is divided into three groups, as follows:

1. The structure and development of the bones of fowls.
2. The kinds of fractures and the reparative processes.
3. Means of controlling the bird and care of the fracture.

I. The Structure of Bone

The bones of the domestic fowl may be divided into four classes, as follows: long, short, flat, and irregular.

Long bones are found in the limbs, where they form a system of levers to sustain the weight of the body and confer the power of locomotion upon it. The femur, tibia, and humerus are examples of this class. The shaft of the long bone is contracted and narrowed to afford greater space for the bellies of the muscles. The extremities generally are somewhat expanded for greater convenience of motor connection, for the purpose of articulation, and for affording a bony surface for muscular attachment. Some long bones are slightly curved, thus affording greater strength to them.

Short bones may be found where a part of the skeleton is intended for strength, with its motion slight and limited, and where it is divided into a number of small pieces, united by ligaments. Here the separate bones are short and compressed. The bones of the toe are examples of short bones.

The patella and the two carpal bones are irregular.

Flat bones may be found where the principal requirement is extensive protection or large bony surface for muscular attachment, osseous structures expanded into flat bony plates, are found, as in the case of the skull and pelvis. In the cranial bones, also, there are layers of compact tissue known as tables. The outer table is thick and tough; the inner table thinner, denser, and more brittle, and, therefore, termed the vitreous table, while the intervening cancellated tissue, which is permeated by air spaces, is called diploe.

Many of the bones of the fowl, as the head, vertebra, and humerus, contain air cavities partitioned off by fine bony structures and into which cavities the air sacs send extensions. The respiratory apparatus of the fowl consists of two lungs, which occupy the upper thoracic region, pushing out between the ribs and composed of a series of tubes. Some of the bronchi or air tubules communicate with air sacs or bladder-like structures located at the anterior thoracic region, others at the diaphragmatic and abdominal regions, and these send extensions into the bones.

Studying more minutely the structure of bone, it is found that it nearly always develops from a connective tissue foundation. The inorganic substance of the bone is compressed in or between the fibers of connective tissue, while the cells of the latter are transformed into bone cells. Between the fibers are uncalcified bone cells, each of which rests in a cavity of the matrix called a lacuna.

Primarily, bone consists of a single thin lamella, its later complicated structure being produced by the formation of new lamellæ in apposition to the first. During its development the bone becomes vascularized and the vessels are inclosed in especially formed canals, known as haversian canals.[1]

The bone cells have processes that anastamose with the processes of other cells. They lie in special canals known as canaliculi.

Appearance of Transverse Section of Bone

The appearance presented by a transverse section of a long bone is as follows: In the center there is a large cavity which in fetal and early chickhood nearly always contains marrow, but later such bones as the humerus contain air spaces. The periphery of the bone is covered with a dense connective tissue membrane, the periosteum. In the young this membrane may be divided into three layers, the outer consisting mainly of rather coarse white fibrous tissue; the middle, fibroelastic tissue, and the inner, the osteogenic layer. The inner membranous layer is vascular and rich in cellular elements. In the adult bird the osteogenic layer has practically disappeared, leaving here and there a few of its cells, while the fibro-elastic layer is correspondingly thicker. A large number of haversian canals containing blood vessels, best observed on transverse section, are found in compact bone tissue. Lamellæ of bone are plainly visible throughout the ground substance and are arranged in the following manner:

There is a set of lamellæ extending parallel to the external surface of the bone, and another set similarly arranged around the inner marrow or air cavity. There are the so-called fundamental lamellæ — known also as periosteal and marrow lamellæ, or outer and inner circumferential lamellæ.[2] Around the haversian canals are concentrically arranged lamellæ, forming systems of haversian lamellæ. There is a system of interstitial lamellæ wedged in between the haversian system of lamellæ. Scattered between the lamellæ are found the spaces known as lacunæ, containing bone cells. It is probable that all lamellæ are in more or less direct communication with each other by means of fine canals or canaliculi. The canaliculi of the haversian lamellæ of bone are composed of fine white fibrous tissue fibrils imbedded in a ground substance in which they are arranged in layers superimposed in such a way that the fibers in the several layers cross at right angles, forming an angle of 45 degrees with a long axis of haversian canals.

In each lacuna there is found a nucleated bone cell which practically fills it, with its processes extended out into the canaliculi.

The haversian canals contain blood vessels, either an artery or vein or both. Between the vessels and the walls of the canals are perivascular spaces bounded by endothelial cells. Into these spaces the canaliculi of the haversian system empty. All lacunæ and canaliculi should be considered as filled by lymph plasma which circulates throughout the bones, bathing the bone cells and the processes.[3] In the fetal skeleton it is found that some bones are formed from a fibro-elastic membrane, such as those forming the roof and side of the skull. Others, such as the bones of the extremities, are formed from cartilage. Thus we find two kinds of ossification, intrafibrous and intramembranous and intracartilaginous.[4]

In the intrafibrous ossification no cartilaginous mould or stage precedes the appearance of the bone tissue. The membrane which occupies the place of the future bone is of the nature of connective tissue, and ultimately forms the periosteum. The outer portion is more fibrous than the internal, in which the cells or osteoblasts predominate. The whole tissue is richly supplied with blood vessels.

Beginning of the Process

At the onset of the process of bone formation a small network of bone spiculæ is first noticed, radiating from the point or center of ossification. A miscroscopic study shows it to consist of a network of fine, clear fibers with an intervening ground substance. These are called osteogenic fibers. They soon take on a dark granular appearance from the deposit of calcareous granules in the fibers and the intervening matrix. As they calcify they are found to inclose some of the granular corpuscles or osteoblasts. The latter form the corpuscles of the future bone, and the spaces in which they are inclosed are the lacunæ. As the osteogenic cells grow to the periphery they calcify and then give rise to fresh bone spiculæ.

The meshes of the network of bone thus formed contain blood vessels and delicate connective tissue crowded with osteoblasts. These bone spiculæ thicken by the accretion of layers formed by the osteoblasts, and the meshes become smaller. Subsequently separate layers of bone tissue are deposited in the periosteum and around the large vascular channels.[5]

In the intracartilaginous ossification we find the primary bone entirely cartilaginous, and in the long bones the process commences in the center (shaft ossification) and extends toward the extremities, which for some time remains cartilaginous. Subsequently, a similar process commences in one or more places in these extremities (epiphyseal ossification), and gradually extend from them. Growth of long bones in length in the fowl takes place from the cartilaginous portion of the ends.

Ossification of Cartilage

The first step in the ossification of cartilage is the enlargement of the cartilage cells, which arrange themselves in rows at a point termed the center of ossification. The matrix in which they are imbedded increases in quantity, so that the cells become further separated from each other. A deposit of calcareous material now takes place in this matrix, presenting a granular and opaque appearance. Here and there the matrix between two cells of the same row also become calcified, and the transverse bars of calcified substance stretch across from one calcareous column to the other. Thus there are longitudinal growths of cartilaginous cells inclosed in oblong cavities, the walls of which are formed of a calcified matrix which cut off all connection between other groups of cells, and thus primary areolar spaces are formed.

During the same time this process is going on in the center of the solid bar of cartilage of which the fetal bone consists, certain changes also are taking place on its surface. This surface is covered by a vascular membrane, the perichondrium, the inner surface of which is in contact with the cartilage, and on which are gathered the formative or genetic cells, called osteoblasts. These cells form a thin layer of bone tissue between the perichondrium and the cartilage. In this first stage of ossification there are two processes going on simultaneously: (1) In the center of the cartilage the formation of a number of oblong processes, and (2) on the surface of the cartilage the formation of a layer of true bone.

Second Stage of Bone Formation

The second stage in the formation of bone consists of prolongation into the cartilage of processes of the osteogenic layer of the perichondrium, which has now become periosteum. The processes consist of blood vessels and cells, osteoblasts or bone formers and osteoclasts or bone destroyers. The latter are similar to the giant cells found in the marrow. They excavate passages through the newly formed bony layers and grow through into the calcified matrix.

Wherever these processes come in contact with the calcified walls or the primary areolæ they absorb them, and thus cause assimilation of the original cavity and the formation of large spaces known as the medullary spaces. These spaces become filled with embryonic marrow, consisting of vessels carrying osteoblasts on their walls, and are derived in the manner described above from the osteogenic layer of the priosteum.

The walls of the medullary spaces are of considerable thickness at this time, but they become thickened by the deposit of layers of new bone in their interior in the following manner:

Some of the osteoblasts arrange themselves as an epithelioid layer on the surface of the wall of the canal. This forms a bony stratum and thus the space becomes gradually covered with a layer of true osseous substance. By the repetition of this process the original cavity becomes very much reduced in size, and at last remains a small circular hole, containing blood vessels and a few osteoblasts. This small cavity constitutes the haversian canal of the perfectly ossified bone. These canals are essential in the regeneration of bone.

Other changes may be observed at the centers of ossification. A similar process has been set up elsewhere and has been gradually proceeding toward the end of the shaft, so that in the ossifying bone all the changes described above may be seen in different stages, from the true bone in the center of the shaft to the hyaline cartilage at the extremity.

II. THE REPARATIVE PROCESSES OF BONE

Fracture of Bones

A fracture may be defined as a sudden dissolution of continuity in a bone. The causes of these in a fowl are:

First, injury or trauma, as may be caused by a blow from a stone or stick or by being stepped upon by a large animal such as a horse or cow, or by a gunshot wound.

Second, muscular action. The effect of this can be seen when it is known that bones are most resistant first to traction, next to pressure, still less resistant to flexion or bending, and least of all to torsion.

External violence may be direct or indirect. In fracture from direct violence the bone is broken at or near the spot where the violence is applied. As a rule, the soft structures surrounding the fracture are more or less injured, and more serious results may follow than in fractures by indirect violence. In fractures caused by this direct violence the bone may be comminuted or fissured and perhaps driven into vital organs, as the liver or lungs, if the fracture be near those regions, or into the brain if it be near the cranial region.

In indirect violence the fracture occurs at a distance from the spot where the violence is applied. The bone usually breaks at its weakest point. The fracture may be rendered compound from the fragments, which often are much displaced and are sharp and irregular, being driven through the soft parts.

External violence is the most common cause of fracture in the fowl. The most common bones that are fractured by this cause are those of the legs, and, next, those of the wings.

Pathologic fractures are those from disease. Disease may also become predisposing causes of fractures from slight violence.

Classification of Fractures

Fractures may be classified as follows:

First. Simple fractures. These are breaks in the continuity of the bone, and the skin is not broken.

Second. Compound or open or complicated fractures. These are fractures where the break is accompanied by a break through the skin and soft parts, extending to the seat of the fracture.

A fracture, whether simple or compound, may be spoken of as:

First, according to their extent, as (*a*) complete, when the bone is broken across; (*b*) incomplete or greenstick, when partly broken or partly bent (often seen in quite young chicks); (*c*) comminuted, when broken into several pieces; and (*d*) multiple, when two or more distinct fractures occur in the same bone or in different bones.

Second, according to the condition of the fragments, as (*a*) impacted, when one fragment is driven into another; (*b*) fissured, when extending through the bone without displacement, or (*c*) infraction depressed, when one fragment is pressed in below the surface, as in some fractures of the cranium; (*d*) punctured, when there is a small perforation with driving inwards of the fragments; and (*e*) splintered, when only a fragment of a bone is chipped off.

Third, according to the line of fracture, as (*a*) transverse, (*b*) oblique, (*c*) spiral, (*d*) longitudinal, (*e*) Y- or T-shaped, and (*f*) stellate.[6] All six of these fractures are recognized.

A fracture is spoken of as "complicated" when associated with other injuries, such as dislocation of the same bone, rupture of the principal artery of the limb, injury of an adjoining viscus, as the brain or liver, or implication of the joints.

Displacements of the fragments often occur. The cause of displacements may be as follows: The violence producing the fracture; careless handling of the injured bird; injudicious movements on the part of the bird; the weight of the lower fragments; and muscular spasms acting on the upper fragments. The amount of these displacements will depend in part on the direction of the line of fracture, and, in part, whether or not the periosteum is torn. The displacement is spoken of as angular, lateral, longitudinal, or rotary.

Symptoms of Fracture

The general signs of fracture in the fowl are unnatural attitude of the wing or leg, inability to use either, alteration in the shape of the part, and a swelling, shortening, or crepitus. In handling the part the feeling of the sense of crepitus is a diagnostic symptom.

If the fractured ends glide past each other, the contraction of the muscles may cause a shortening of the limb. The muscles of the fowl are loosely connected by fascia and displacement of the fractured ends of the bone is common. Pain usually accompanies a fracture in the bird.

So-called fracture fever may occur in the case of a break of the femur. This elevation of temperature may last for a day or two. It is supposed to be due, at least in part, to the absorption of tissue products.

Process of Repair

The union of fractures by callus is similar to that which takes place in the healing of the wound of the soft parts by first intention. Blood is at first extravicated between and around the fragment until the ruptured or torn vessels are closed by clot. Within twenty-four hours after the injury there begins a simple inflammation from the torn vessels of the bone, the periosteum and the surrounding soft parts. This process includes an emigration of leucocytes and an exudation of fluid.

Then commences repair. The cells of the osteogenic layer of the periosteum begin to proliferate, and to a less extent the same is true of the bone corpuscles and surrounding endothelial and connective tissue cells. These cells gradually infiltrate, remove the clot, and collect to form the temporary callus. From the second day onward, cells (phagocytes) are seen containing remains of leucocytes, red blood corpuscles, and tissue fragments. In a simple normal fracture there is no emigration of leucocytes or pathologic exudation after six days.

The mass of soft, red, gelatinous granulation tissue is composed of tissue cells, derived from the periosteum, bone, and connective tissue, which are similar to the fibroblasts in a wound, with the addition that cells, especially those from the periosteum, have osteogenic properties, are osteoblasts.[7] Between these cells newly formed capillaries grow in from the vessels in the haversian canals, periosteum, and neighboring connective tissue. The formation of new bone in the callus is like the development of bone in membrane. There is, in addition, a variable amount of a firm, gelatinous intercellular substance, which when in considerable amounts distinctly separates the cells, and gives a bluish appearance to the naked eye, and is called cartilage. It appears that when the fractured ends of the bones are kept at rest and in perfect apposition, and with a normal rate of callus formation, the process is one entirely of formation of bone in membrane, without any preformed cartilage; but, on the other hand, if the bones are not in proper apposition movement of the ends and a delayed formation of callus, islands or masses of cartilage may appear.[8]

The osteoblasts from the periosteum give rise to the ensheathing callus and to the definitive callus, especially in the case of long bones. The callus is found replacing the periosteum, and extending for some distance around the bone both above and below the line of fracture, forming a spindle-shaped tumor, by which the ends of the fragments are surrounded. It also replaces the structures within the canal by similar substance forming the provisional plug or internal or endosteal callus; and between the ends of the fragments the permanent intermediate or definitive callus.

The ensheathing callus and the internal callus are gradually organized into fibrous tissue, becoming harder and firmer. The outermost layers of the fibrous tissue into which the ensheathing callus is thus converted form a new periosteum. Ossification of the ensheathing callus is given by Spencer and Gask as beginning on the twelfth to the fifteenth day in the human,[9] but in fowls it is much earlier. This process usually begins in the angle between the periosteum and the bone, and extends along the surface of the bone, and also along the surface of the ensheathing callus beneath a new periosteum, till the upper and lower layers of the ossifying callus meet opposite the line of fracture.

Ossification of the internal callus goes on in a similar way, but begins a little later. The permanent callus, as soon as the ends of the bone are thus fixed by the ensheathing and internal callus, also undergoes ossification. The ossified callus is at first very vascular and porous, and can easily be stripped off the old bone, but later it becomes hard and dense, through formation of new bone around its blood spaces, and is then intimately connected with the old bone beneath it. Finally the ensheathing callus and internal callus, having completed their function, are gradually absorbed, and if the fragments have been held in good apposition no sign of the fracture may ultimately remain. Spencer and Gask give the time for such processes as being complete in the human at six or eight weeks, and that many months elapse before repair can be spoken of as complete.[5] In the fowl these processes are much more rapid.

The process of absorption of the temporary callus consists in the removal of the lime salts, leaving fibrous tissue, which in turn undergoes absorption, so that muscles, tendons, and nerves involved in the callus become freed and regain their function.

The Healing of Improperly Set Fractures

Where the bones are not in exact apposition a modeling process occurs which may transform much of the internal structure of the bone.

The course of the septa, whether in persisting ensheathing callus or in the bone itself, takes a changed direction in accordance with the altered transmission of weight through the bone and the different angle at which the muscles pull.

Where the ends of the fragments overlap, the ensheathing callus fills up the angles; and while the open end of the medullary canal in each fragment in thus closed, its continuity through the bone is restored by the absorption of the intervening walls of the contiguous and overlapping fragments. Where the fragments are not in contact, the intervening space becomes filled with the ensheathing callus, which is then sometimes called interposed callus. Where the fracture is comminuted, the splintered fragments become glued, as it were, together, and to the main fragments by the ensheathing callus formed from the vascular tissue in which they become surrounded. When the fragments are in good apposition and are kept relatively at rest little ensheathing callus is found; but where there is much displacement, or where rest is impossible, as in a fracture of a rib, or difficult to obtain, as in a fractured clavicle, a considerable amount is produced.

The series of studies here presented are of two kinds: First, a study of the reparative processes; second, a study of the means of control of the fracture and care of the bird.

Figure 1 illustrates the study of a series of fractures that have been repaired for a considerable length of time. *A* shows a repaired ulna which has sustained an angular break, with a sliver of bone split off of the upper side and extending nearly to the proximal end of the bone. *1* shows the repair and a porous provisional callus. At *2* is shown that the reparative bone material has been lavishly used. Letter *C,* Nos. *6* and *7,* show the same. Letter *B* shows a tibia and fibula of a hen which has sustained a fracture at right angles to the shaft of the bone—(*3* is the fibula; *4* the tibia; *5* the repaired fracture). That this fracture has been repaired a long time is indicated by the fact that practically all of the provisional callus has been removed by absorption. Letter *D* shows the bone at a different angle, and shows at *8* the fibula, at *9* the tibia, and *10* the repaired fracture. This bone was improperly cared for, and hence a crooked leg was the result.

A series of studies were made of the nature and rapidity of repair of fractured bones of a domestic fowl. The birds were chloroformed and the bones fractured and set while the birds were under the anesthesia. The metatarsus and ulna were selected. The material used to hold the bones in place were cotton, one-inch cotton cloth bandage, wooden splints, and glue. At the end of each experiment the bird was again chloroformed and the bone removed. After a physical examination of the bone it was sectioned longitudinally, photographed, and the lesion of one-half cut out and placed in 10 per cent hydrochloric acid solution for forty-eight hours for decalcification, and then passed through three changes of absolute alcohol, and then through equal parts of alcohol and ether, then imbedded in histoloid and sectioned. The sections were stained in hematoxylon and eosin and clarified in oil of cedar and mounted in natural balsam for study. Figure 2, letter *A*, shows a sectioned surface of a metatarsus of a Single-Combed Rhode Island Red cockerel eight days after the fracture. There was a mottled reddish white zone in the region of the fracture, indicating that immediately following the fracture there was an extravasation of blood which had collected around and between the fragments and between the ends of the compact portion of the bone, and had also invaded, to a certain extent, the marrow cavity. The fluid at this time gave some evidence of advanced organization and was rather callus-like, but allowing the fractured ends of the bone to separate when traction was applied.

There was present the initial hyperemia of repair. This hyperemia was most marked in the periosteum. Leucocytes, whose function it is to digest and remove the detritus resulting from the injury, had invaded the parts. Proliferated changes had taken place in the connective tissue, and in fact this was observed in cases of only forty-eight hours standing. The most active cellular multiplication was in connection with the fibrous structure of the periosteum. This forms the germinative or reparative tissue from which arises the osteoblasts. The nature of the new formed structure was that of connective tissue, and in Figure 4 may be seen the commencement of this organization into trabecular-like arrangement forming the periosteal callus and the provisional plug. It can be seen that this has been poured out and formed from the periosteum. This field shows many fibroblasts and is packed with osteoblasts and osteoclasts, and in still other fields of the trabeculæ a homogeneous matrix with formative bone cells in their lacunæ. The repair is apparently one of intramembranous bone formation, with islands of new formed bone at the end of the fifth day.

In Figure 2, Letter *B*, is seen a sectioned surface of a fractured metatarsus of thirteen days standing. This bone is from a one-year-old Single-Comb White Leghorn hen, who was of low vitality and the reparative processes were more tardy than in section, letter *C*, shown in the same cut. After the metatarsus was removed it could with considerable force be made to spring, which was not the case in *C*, where the same amount of force was used. Both birds showed the reparative processes far enough advanced to have the appliance or cast removed with safety.

From these two studies it is rather indicative that repair of the bone of the domestic fowl is quite rapid and that twelve days is ample time to allow the bandage or cast to remain in place.

Figure 5 shows a photomicrograph of a section of the fractured portion of the bone shown in Figure 2, letter *C*. Figure 5 at 2 shows the periosteal or ensheathing callus, at 3 the internal or endosteal callus, and 4 the intermediate or definitive callus. A comparison of the calluses in Figures 4 and 5 show at a glance that the formation of bone in Figure 4 is just commencing to near completion in some of the trabeculæ, while in Figure 5 the process is nearly completed. Newly formed bone cells are plainly visible in the photomicrograph.

Figure 3 shows a series of studies at different ages. No. 1 shows the ulna of a Single-Comb Rhode Island Red cockerel nine months old. This fracture was of eight days standing. The left-hand view shows the provisional callus which appeared irregular in outline but smooth surface and whitish and pink mottled, indicative of a normal reparative process. The right-hand view shows the same in inner section. The process showed in the eight-day specimen marked advancement of complete normal bone tissue over the five-day specimen, but the firmness of the fractured end was not such that the bandage could have been removed safely. Number 2, in the same one-year-old Single-Comb White Leghorn hen, shows the process at the eighth day in a similar stage to the previous case. No. 3 of the same cut shows a case at fifteen days standing.

Numbers 4 and 5 show two cases of the tibia of a Buff Plymouth Rock hen in which the muscles of the tibial region had pulled the fractured ends past each other; thus a vicious repair was the result. It will be noted that the ensheathing callus has filled in the angles so that the fractured portion presents an irregular roundish appearance.

The ninth and tenth cases were Buff Orpington and Single-Comb White Leghorn respectively. The ulna was fractured and allowed to proceed ten days in its reparative process.

III. The Treatment of Fractures and Care of the Bird

Following the definite diagnosis of fracture of a bone in the fowl, the next step is to detremine if any wound has been made which extends through the flesh and skin.

In simple or so-called subcutaneous fracture the fragments of bone should be placed in perfect apposition and the normal shape of the bone restored. The loose arrangement of the muscles makes this an easy task.

The next step is to apply apparatus holding the parts firmly in place until firm union has taken place. Spasmodic contraction of muscles is not likely to be encountered except in the thigh, arm, or breast muscles. In applying the apparatus, normal functions, as circulation and nerves, must be safeguarded. After the setting is complete, the bird must be provided with a clean coop and grassy run where other birds will not interfere. Good food and water and an occasional examination to determine if all is well with the bone undergoing repair is also needed.

Setting of Fracture

If the fracture is on a feathered part, the feathers in the region to be manipulated should be removed. Next, apply a thin layer of cotton, holding carefully the fractured part in the proper position, then apply about three thin, narrow wood splints (somewhat shorter than the fractured bone) in such a manner as not to later chafe the skin and make a sore, and, then apply one-inch cotton bandage, applying at the same time glue or laundry starch. In a few hours the glue or starch (preferably glue) will be dry and the parts held firmly in position. Plaster of Paris may be used in the place of glue, but the cast will be found to be rather heavy for a small animal like the fowl.

At the end of twelve or thirteen days carefully remove the appliance, dissolving the glue or starch in warm water. Confine the bird for a few more days longer in a separate compartment.

In feeding for the first two or three days it is well to feed mash or bread soaked in milk, and then gradually feed corn, wheat, and oats, as for other fowls, and give milk or water to drink. If there are no internal injuries or other complications the birds will not greatly suffer.

It is found that in fractures near the joint the new bone thrown out in reparative processes may result in a stiff joint. Where the joint is stiff it is found that the tendons are more or less adherent to each other and to the fractured part, and at times they may be atrophied. Muscles of the bird which are not brought daily into use soon undergo atrophy.

One of the unfavorable outcomes of a fracture is a false joint or pseudoarthrosis in which is had an ununited fracture of some standing in which the ends of the fragments are rounded off and eburnated, or covered with a layer of fibro-cartilage, and inclosed in a strong fibrous capsule formed by the condensation of the surrounding soft tissue. If the fracture is near the articular ends of bones where a rotary as well as angular movements take place, the false joint may resemble a ball and socket joint, or it may resemble a hinge joint. If the fracture is through the shaft of a bone, and angular movements alone take place, the false joint is likely to resemble a hinge joint. Some of the more common causes for these false joints are: The fragments not having been kept thoroughly at rest; the fragments not having been kept in perfect apposition, as a sequala of muscular contraction; the loss of large pieces of bone, as in compound fractures; the intervention of a piece of tendon, muscle, periosteum, or bullet, as in gunshot wounds and fractures; and the effusion of synovial fluid in the case of fracture into joints.

False joints may be the result of necrosis of the ends of fragments, the poor supply of blood to the fragments, defective nerve influence, as may occur in injury of the spinal cord, malignant growths, and osteomalacia.

Compound fractures or open fractures are those where there is a wound through the skin and other soft structures extending into the fracture. This form may be produced in different ways. The violence causing the break of the bone may be sufficient to tear open the soft tissue, or a fragment may be forced through the parts, or the bird in its movements after the accident may force one of the fragments through the soft structure and the skin. Ulceration may take place later in a bruised or injured part directly over the fracture. This necrosis is preceded by congestion, then inflammation and molecular death.

A wound in compound fracture may be large or may be nothing more than a small puncture. In the case of gunshot wound, and where large animals have stepped on the limb, the bone may be badly crushed. If the wound be a small puncture, with no infection, and the bird is greatly resistant to infection, the repair may take place as in simple fracture. When the wound is large and infection has taken place (which infection may be septic), and there has been laceration of the soft part (or comminution of the bone), union is affected by granulation from the ends of the fragments and periosteum, the process being similar to union of soft parts by second intention or by granulation. The loose parts and fragments are cast off by sloughing. If the fragment is in connection with the periosteum and receives nourishment, it may be retained. If the portion of the bone be denuded of periosteum, that part usually undergoes necrosis and is cast off. If it is not properly cast off, and is surrounded by new bone, it may remain a source of irritation. Some of the dangers of these compound fractures are necrosis of bone, gangrene of the limb, and septicemia.

In applying the bandage to an open fracture it is best to place a small piece of cork or wood over the wound and, after applying the dressing, cut down through the dressing and remove the cork. In this way a drainage window is established and through this the wound may be treated daily with an antiseptic wash.

Vicious union is a condition which results where the leg has been improperly set or the bandage removed too early and the weight of the bird causing the bone to unite at an angle. In this case deformity of the limb is the result.

A series of cases were studied from a practical treatment standpoint. Figure 6 is a röntgengraph showing a normal femur. Figure 7 shows a röntgengraph of a properly set fracture. Figure 8 is a röntgengraph which shows an improperly set tibia. This is of three days standing. This fracture, if allowed to remain in this condition, would have made a vicious union and a deformed limb, which in a show bird is a serious defect.

Figure 9 shows a fracture six days standing and Figure 10 is a röntgengraph of the same fracture at nine days standing. It will be noticed that the callus is becoming more dense, due to the increased molecular density brought about by the reparative process. Figure 11 shows the same fracture twelve days standing.

Figure 12 shows a plaster of Paris cast which was used on case No. 15. These casts are rather heavy for birds.

Figure 13 shows a fracture with adjustment and appliance in place. This is a fracture of a tibia in a three-pound Single-Comb Rhode Island Red pullet. The appliance consisted of cotton, wooden splints, one-inch cotton bandage, and glue. Glue has proven the most satisfactory in the experience of this laboratory.

Figure 14 is the last of the series of twenty-one birds studied. *A* shows a normal shank and *B* a vicious union, with deformity. This is similar to numbers 3 and 4 in Figure 3. It was a Buff Plymouth Rock cock, which was rendered blemished for show purposes due to improper care of the fracture.

In one case where the bones of the wing were not in perfect apposition, and not securely held, the bird was handled often and the ends of the bone moved frequently, there was found a few islands of cartilage. This was not observed where the broken ends of bone were firmly held in proper position.

SUMMARY

A series of twenty-one cases of fractures were studied in the domestic fowl. It was found that at the end of the fifth day islands of bone tissue had begun to form.

The repair of fractures in the domestic fowl is intramembranous.

The periosteal, endosteal, and intermediary calluses show bone formation in trabecular-like arrangement.

By the end of the thirteenth day the major portion of the bone tissue had formed and was found completed before the twentieth day.

The appliance used to hold the broken bones in apposition in the domestic fowl may be removed with safety by the end of the twelfth or thirteenth day.

The structure of compact bone in the domestic fowl is similar to that of mammalia.

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DESCRIPTIONS OF THE ILLUSTRATIONS

FIG. NO. 1—*A*, a broken ulna. *1* shows the diagonal fracture, with repair complete. Note the porous bony mass. *2*, a splintered piece of bone which has been united to the main bone in the reparative processes.

B, a broken tibia which has been repaired. *3*, the fibula. *4*, the tibia, *5*, the point of fracture.

C, a side view of *A* (smaller magnification).

D, *8*, the fibula. *9*, the tibia. *10*, the point of fracture. The provisional callus has been largely absorbed.

FIG. NO. 2—*A*, a fracture of the metatarsus five days standing. Properly set. From a three-year-old S. C. Rhode Island Red hen. *1*, the fracture. At this stage the exudate had not fully organized.

B, fracture of the metatarsus thirteen days standing. From a one-year-old S. C. White Leghorn hen. Hen rather low in vitality. *1*, the provisional callus. *2*, the provisional plug. *3*, the normal metatarsal wall at break. The bone shows a slight spring upon the application of considerable force.

C, fracture of metatarsus thirteen days standing. From an eight-months-old S. C. Rhode Island Red cockerel. *1*, the provisional callus at the point of fracture. *2*, the provisional plug. *3*, the normal metatarsal wall at point of the break. There was no spring when force was applied to this bone.

FIG. NO. 3—*A*, photograph showing the outside surface of fractured bones.

B, photograph showing the inside surface of fractured bones. *1*, fracture eight days standing. *2*, fracture eight days standing. *3*, fracture fifteen days standing. *4* and *5*, fracture twenty-one days standing. The latter two and first are vicious union, the second and third are properly set.

FIG. NO. 4—A photomicrograph of a section of the wall of the metatarsus of a cock. Fracture five days standing. *1*, splintered fractured end. *2*, the provisional callus. *3*, the provisional plug. *4*, the bone cells of the normal bone. *5*, the endosteum. *6*, the haversian canals.

FIG. NO. 5—An oblique fracture of thirteen days standing. *1*, the fractured ends which have been placed in perfect apposition in the process of setting. *2*, the provisional callus. *3*, the provisional plug. *4*, intermediate callus. *5*, the periosteum, one of the sources of the new bone cells.

FIG. NO. 6—A röntgengraph of a normal tibia.

FIG. NO. 7—A röntgengraph of a fracture. Properly set.

FIG. NO. 8—A röntgengraph of a fracture of three days standing. Not properly set. Note the appearance of the provisional callus filling in the angles around the fracture.

FIG. NO. 9—A röntgengraph of a fracture of six days standing (same case as in NO. 8). Note provisional callus.

FIG. NO. 10—A röntgengraph of a fracture of nine days standing (same case as in No. 8). Note the increased density of the provisional callus. The density of the shadow depends upon the molecular density of the tissues. The molecular density of the callus becomes greater as reparative processes advance.

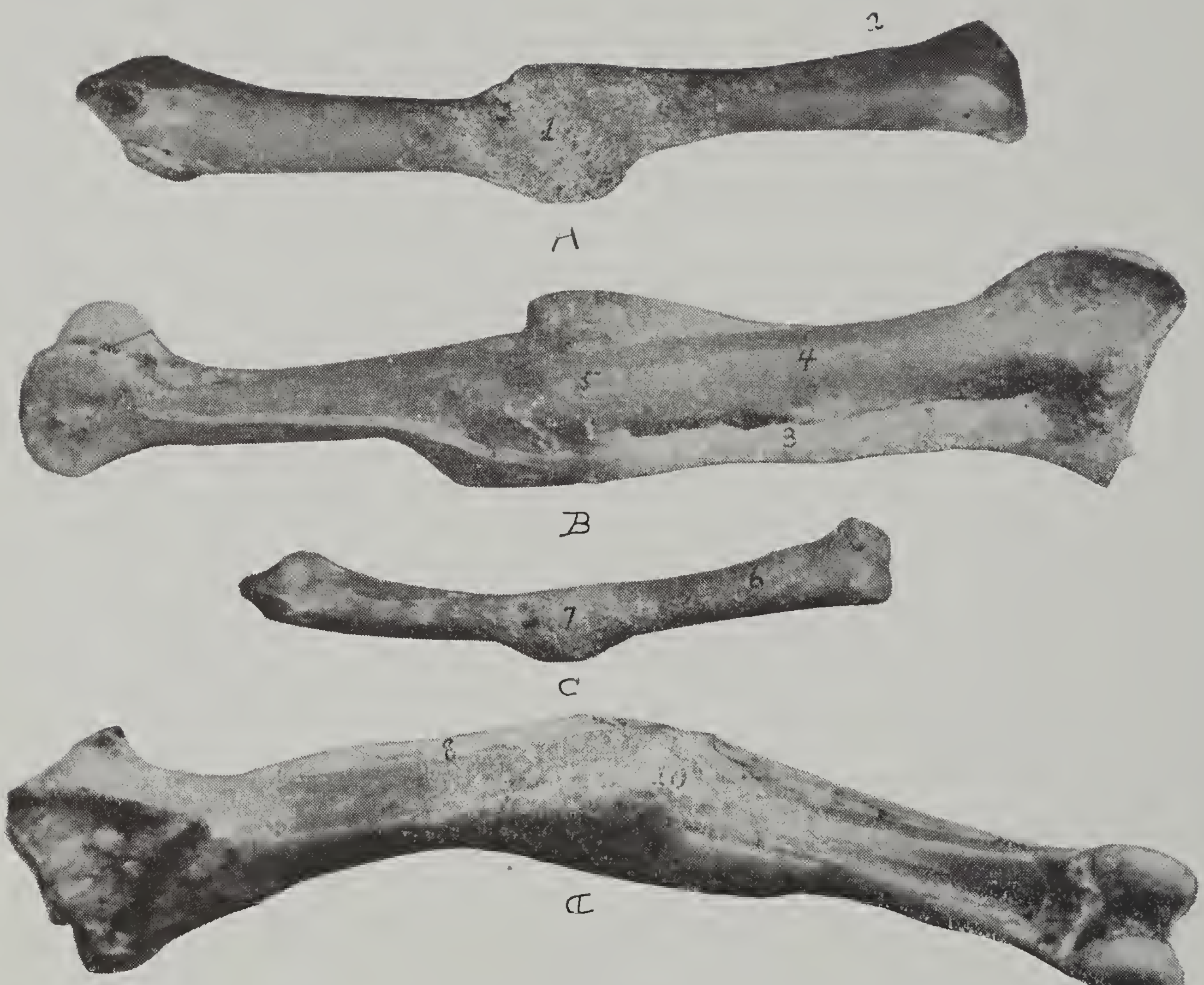
FIG. NO. 11—A röntgengraph of the same case at twelve days standing.

FIG. NO. 12—A plaster of Paris cast from a hen with a broken tibia. Slightly enlarged. Weight 55 grams.

FIG. NO. 13—A four-months-old S. C. Rhode Island Red pullet with a broken tibia. Properly set, using splints (No. 1), cotton (No. 2), one-inch cotton bandage (No. 3), and glue.

FIG. NO. 14—*A*, a normal metatarsus of a cock.

B, a repaired fracture showing an improperly set bone and a vicious union.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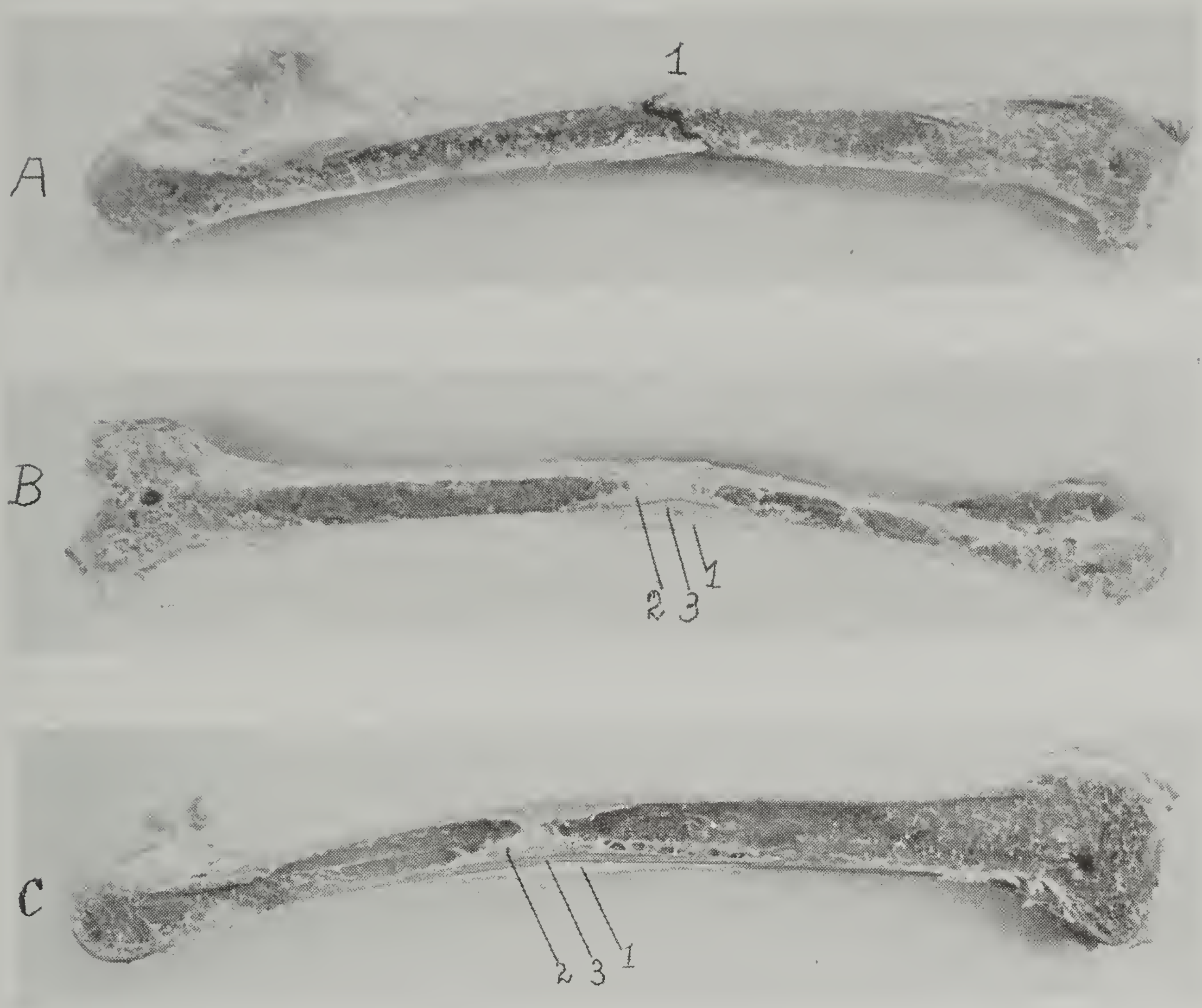
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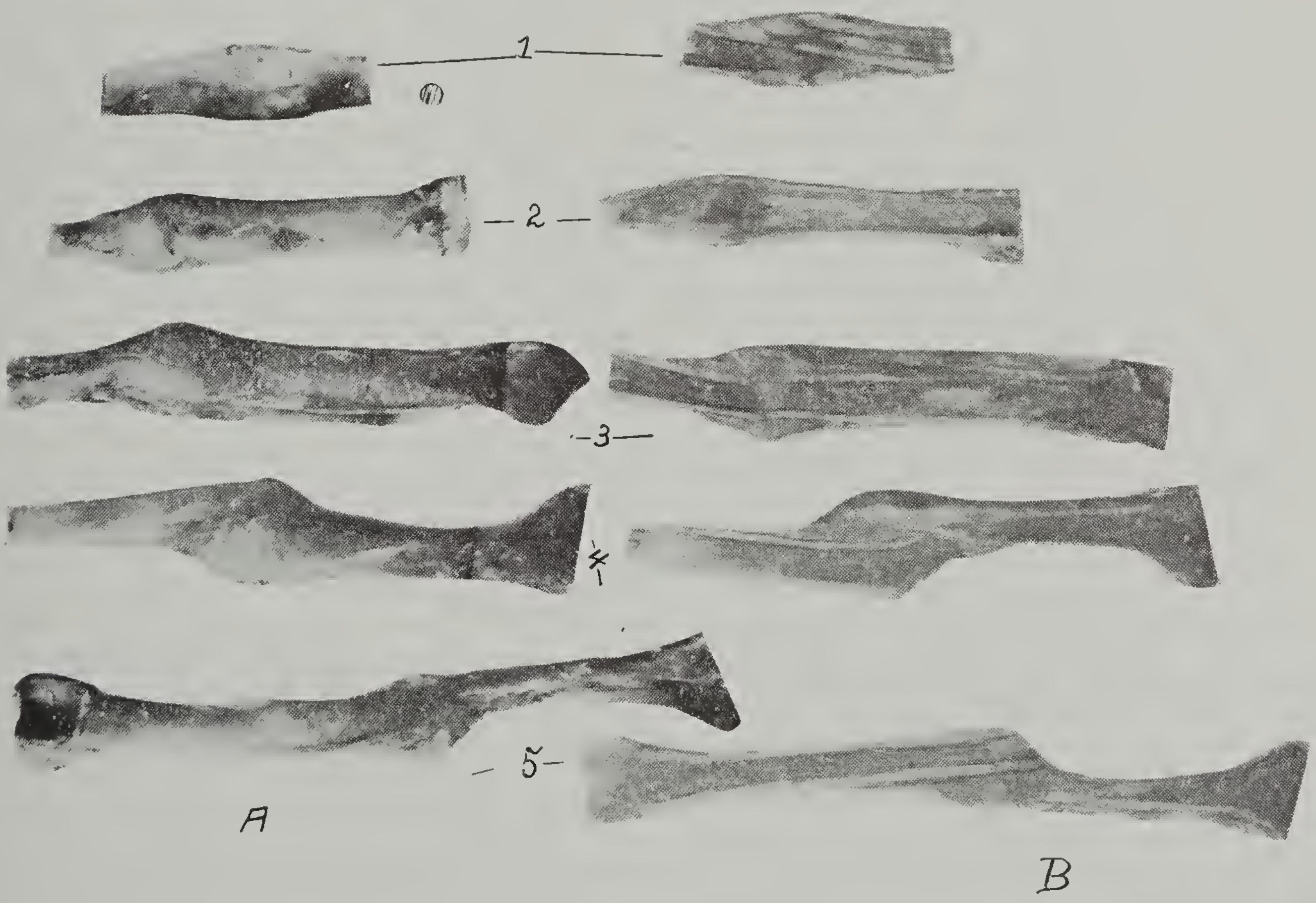

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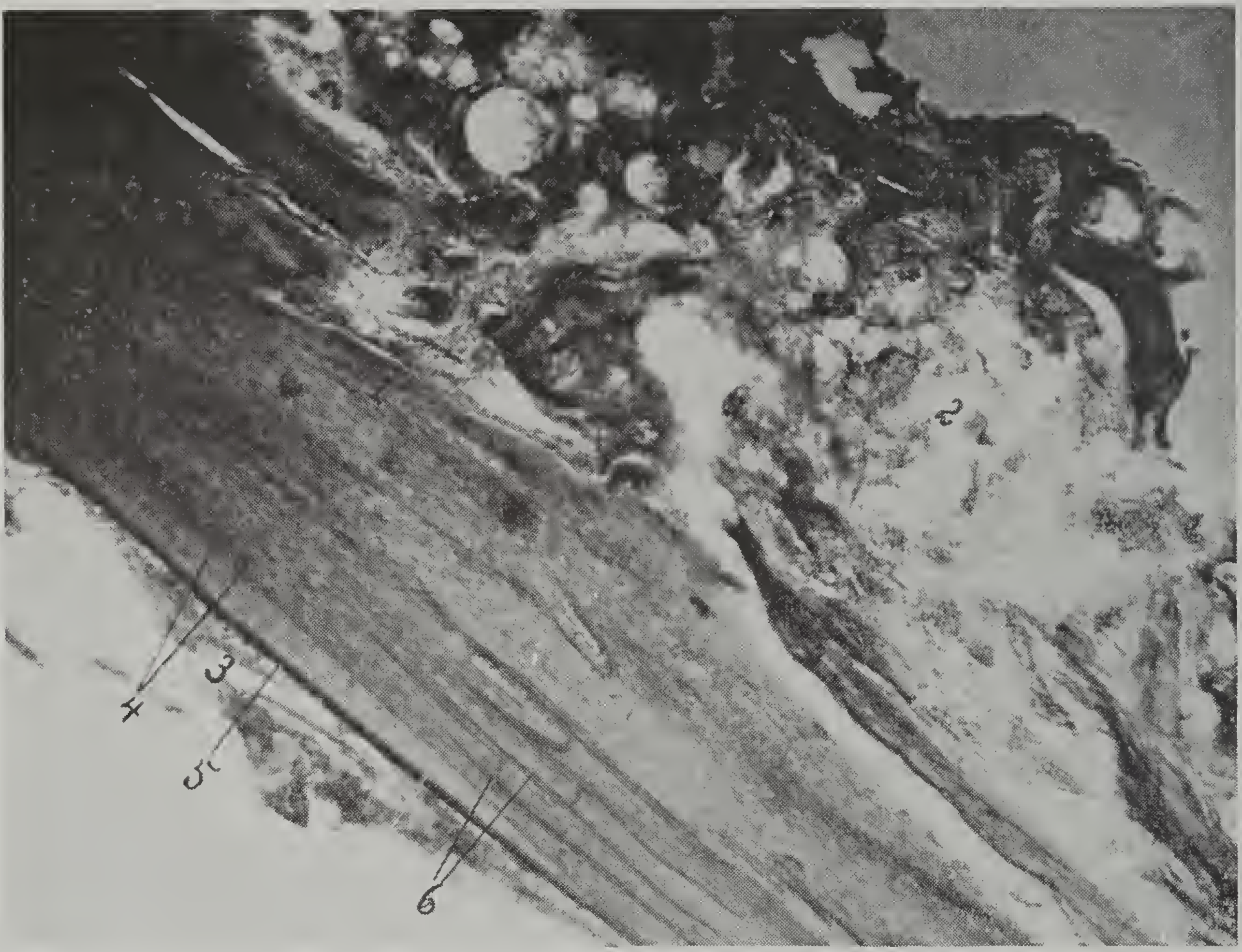

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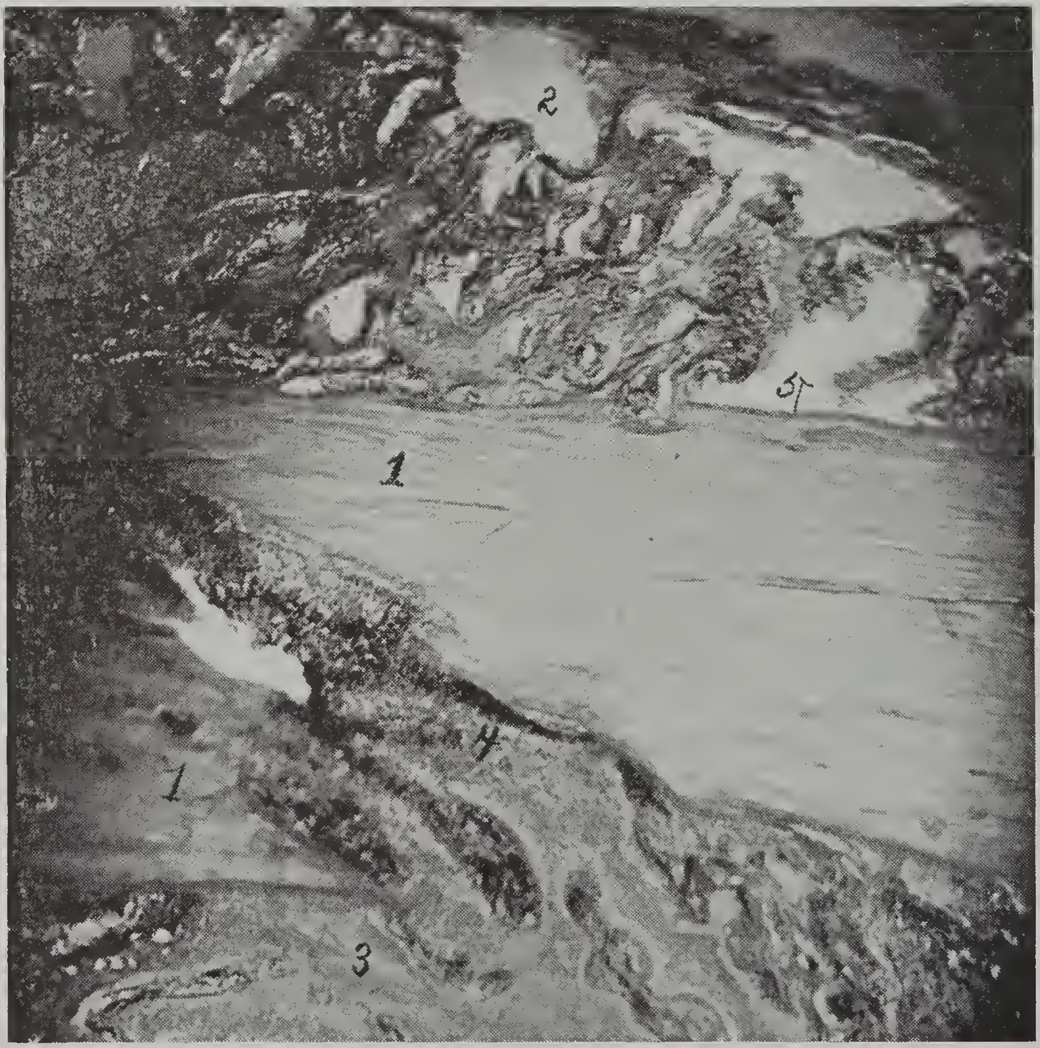

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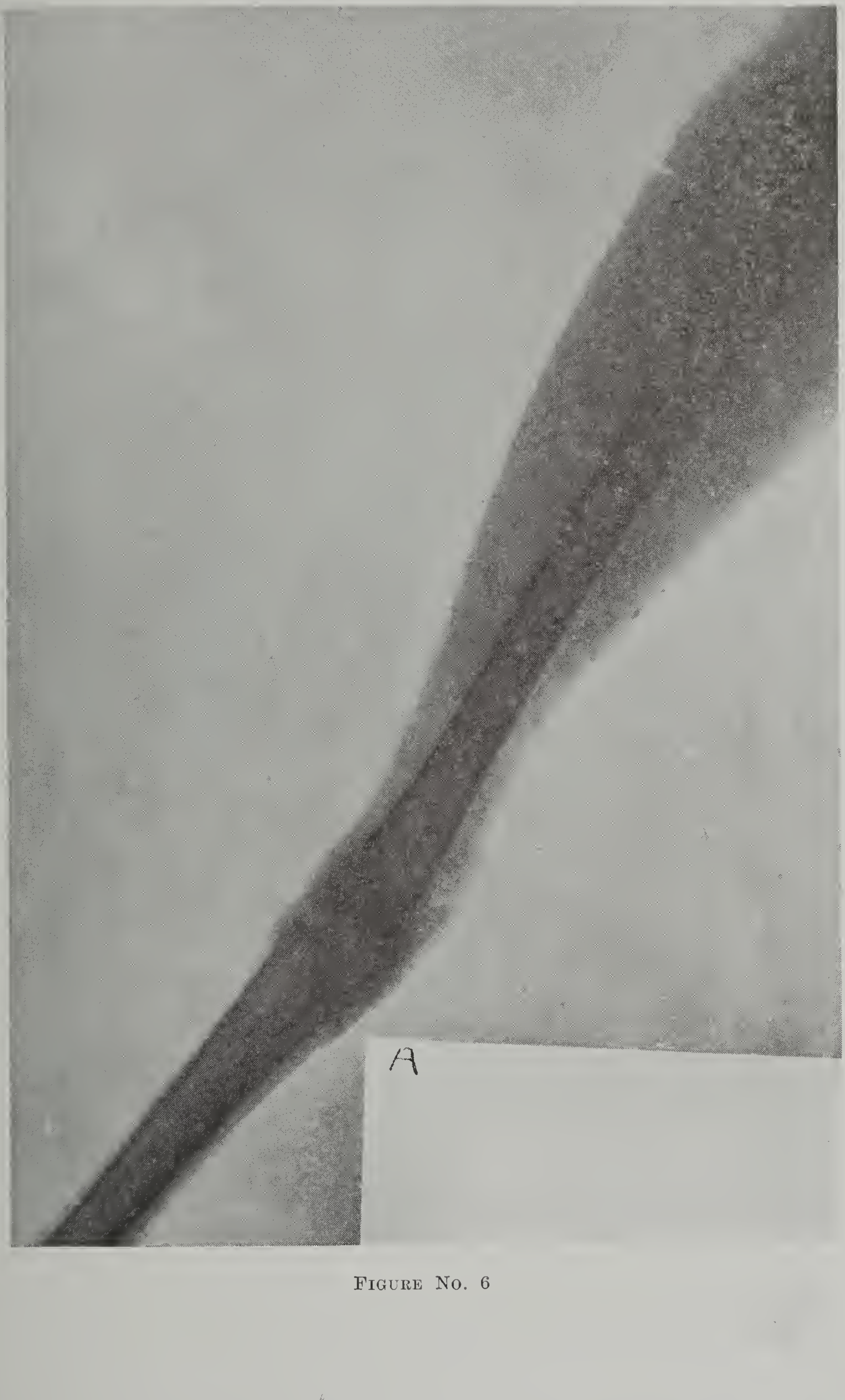

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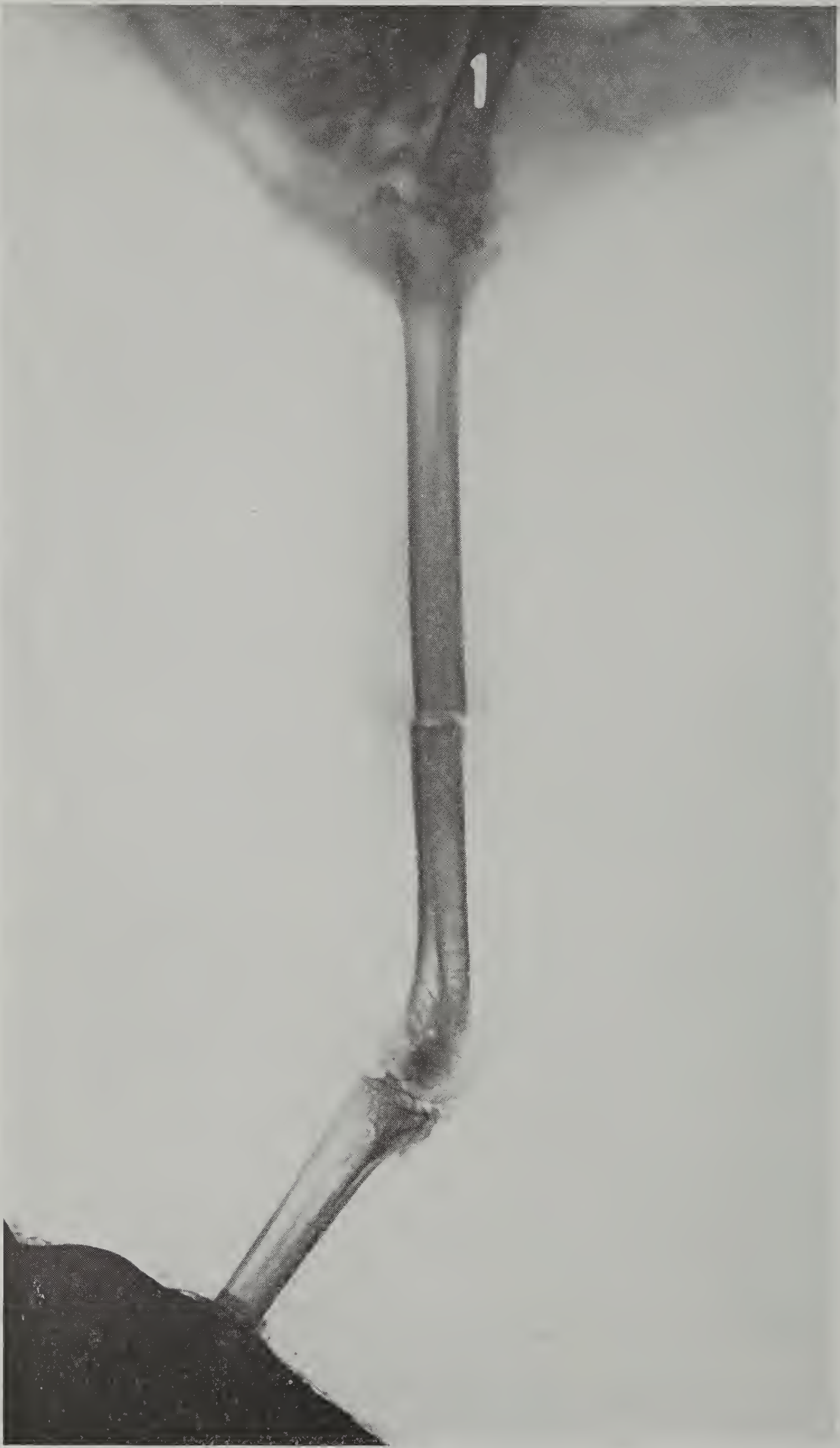

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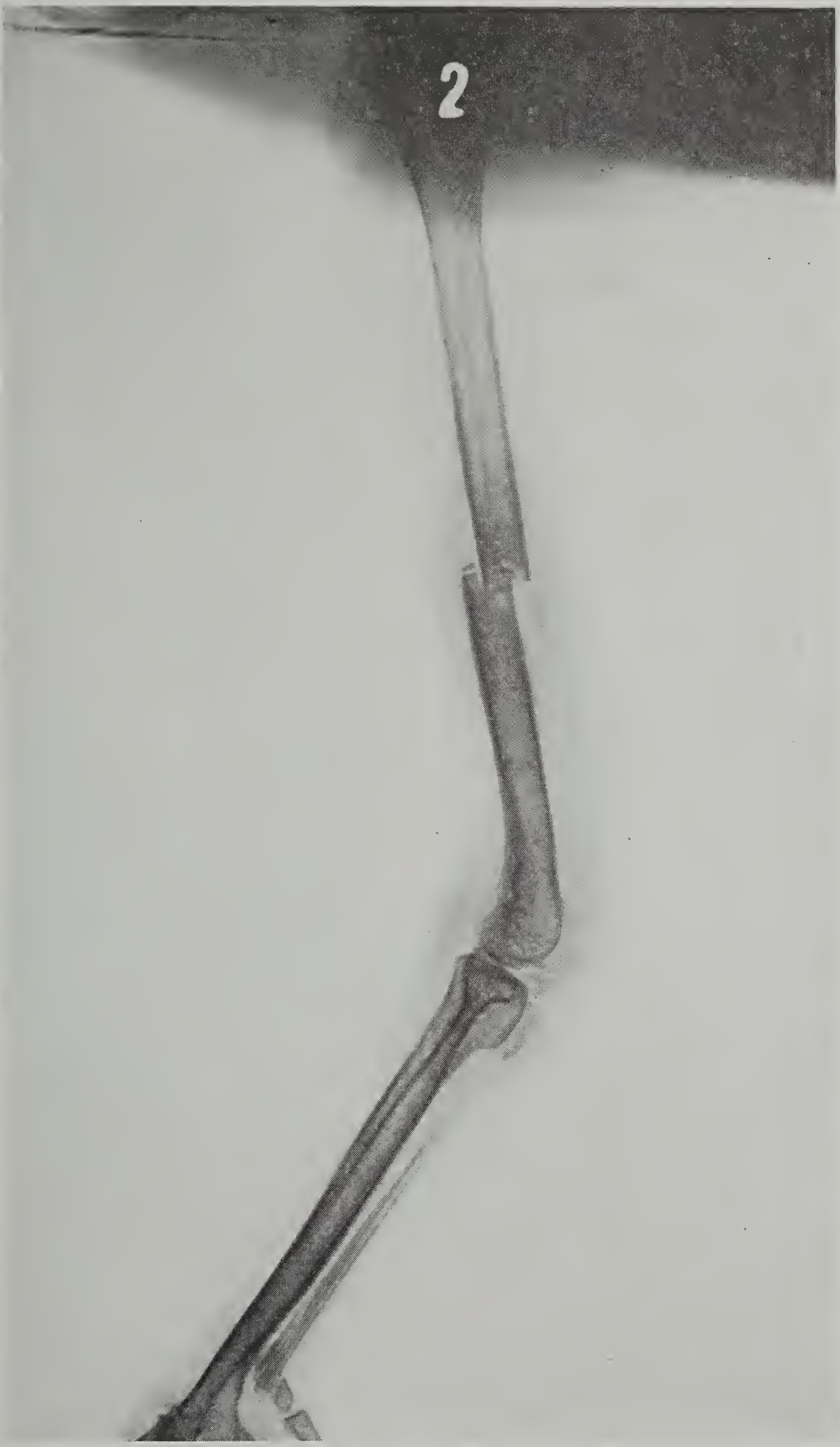

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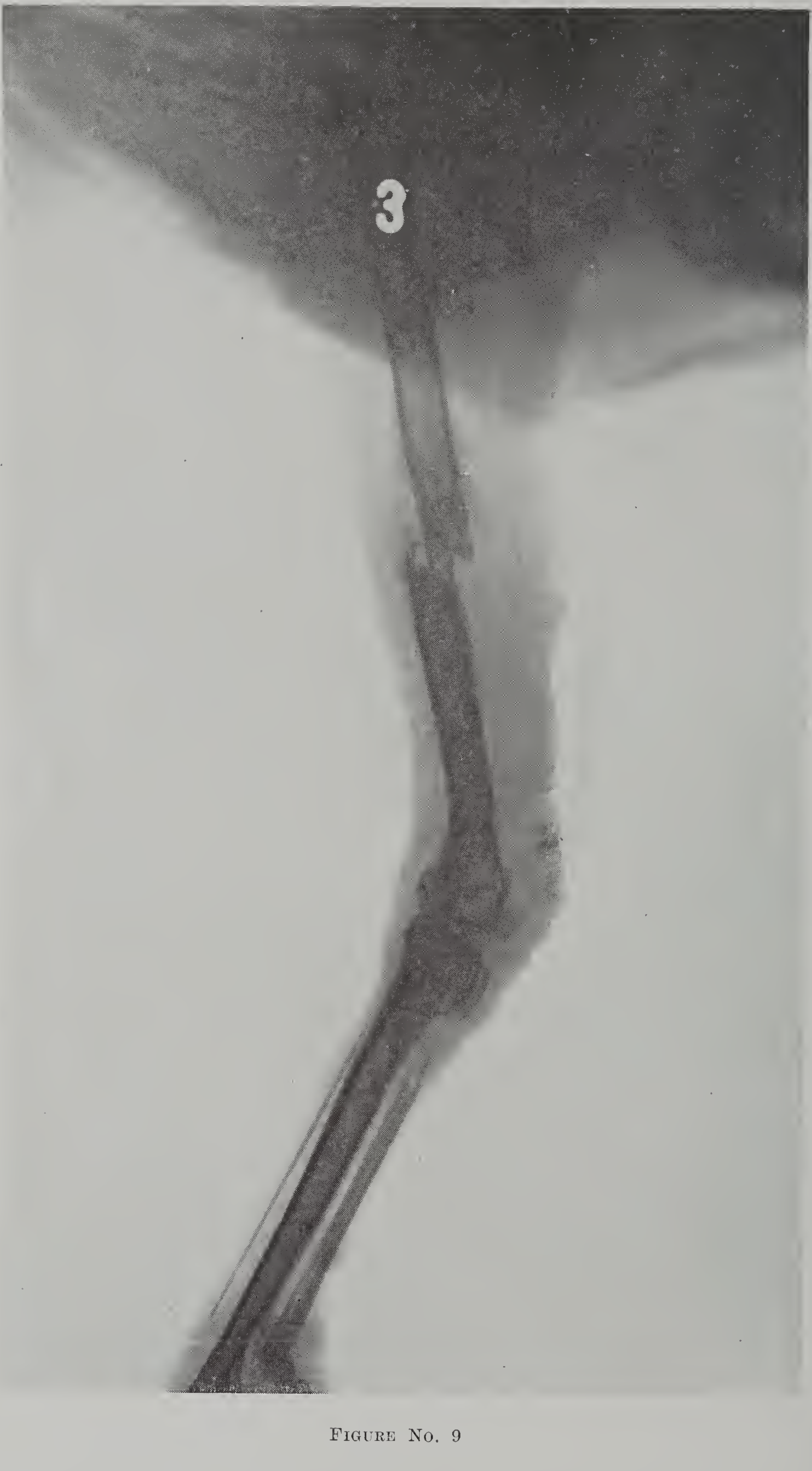

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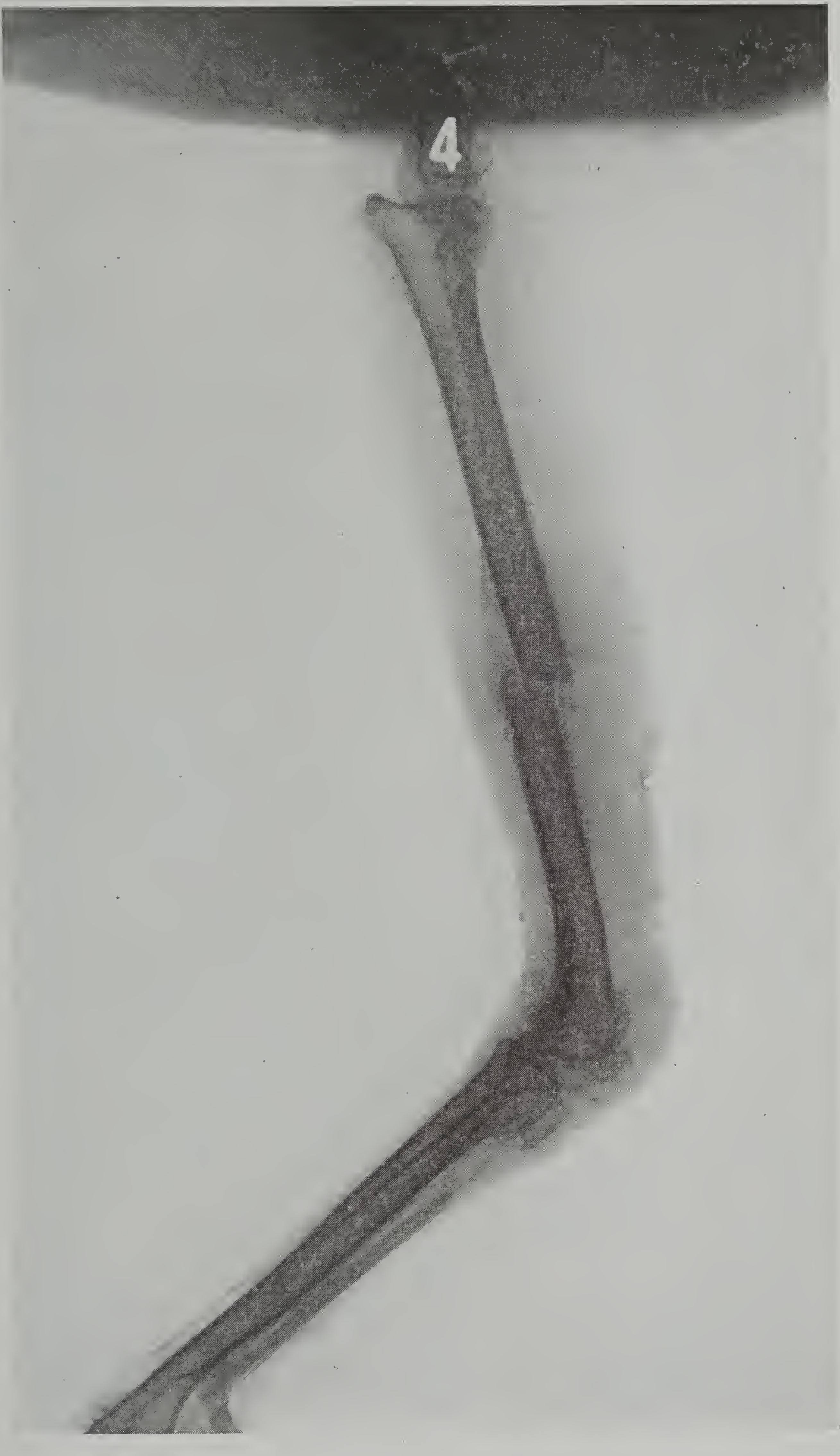

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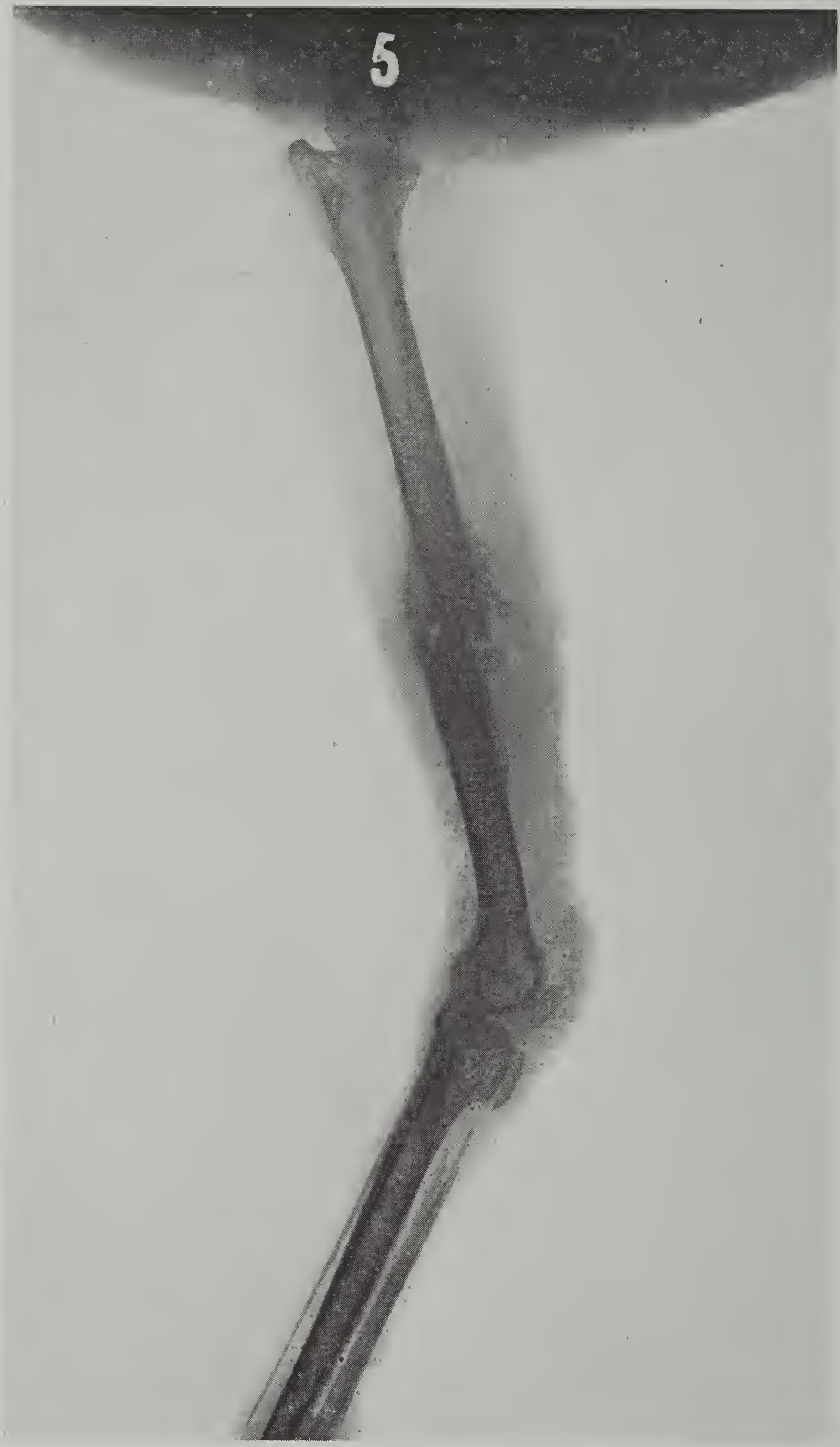

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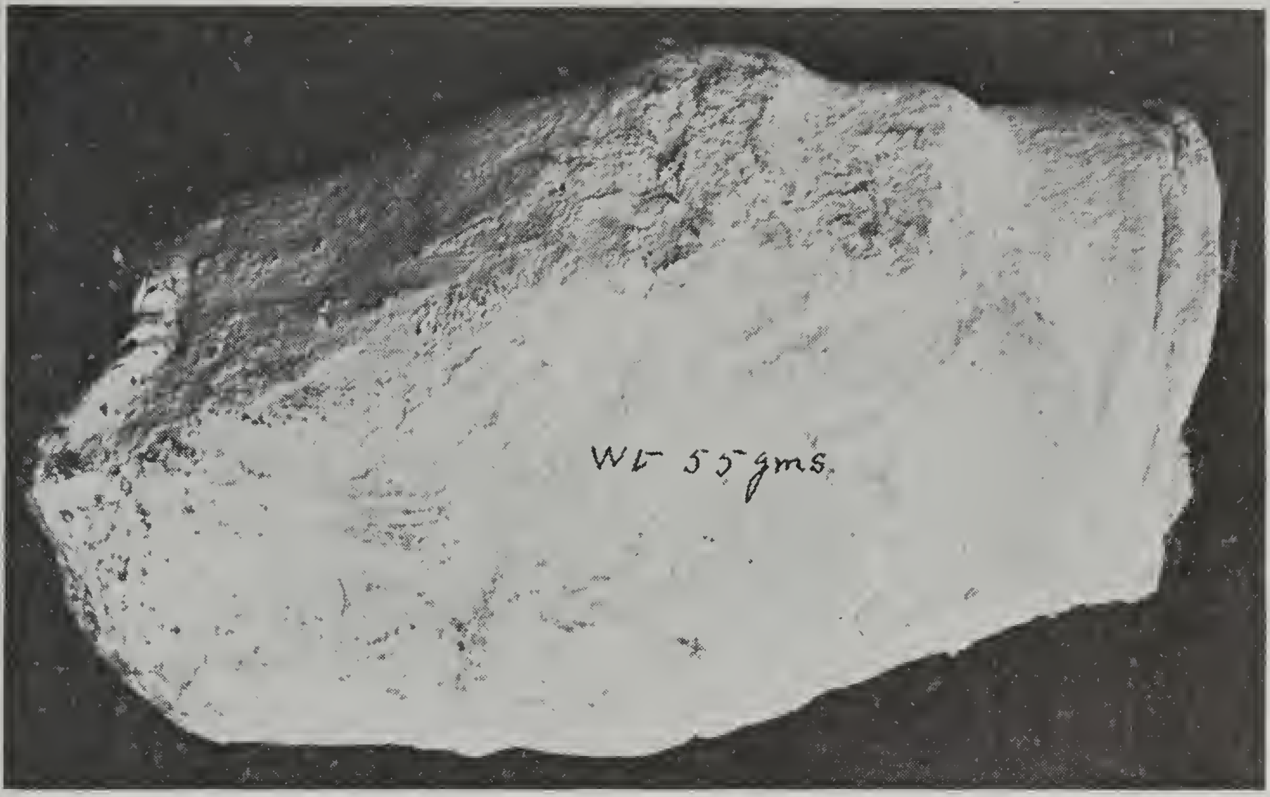

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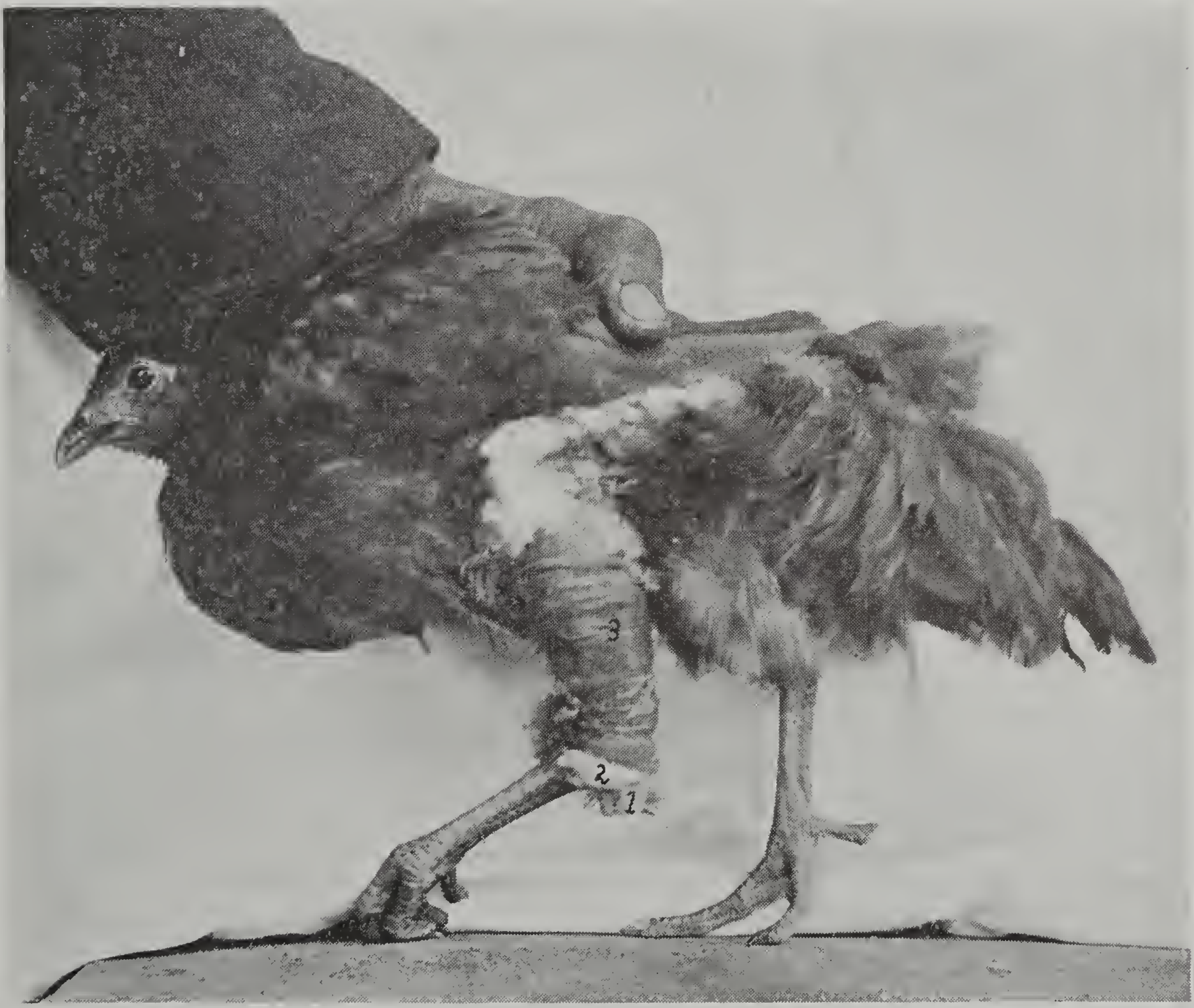

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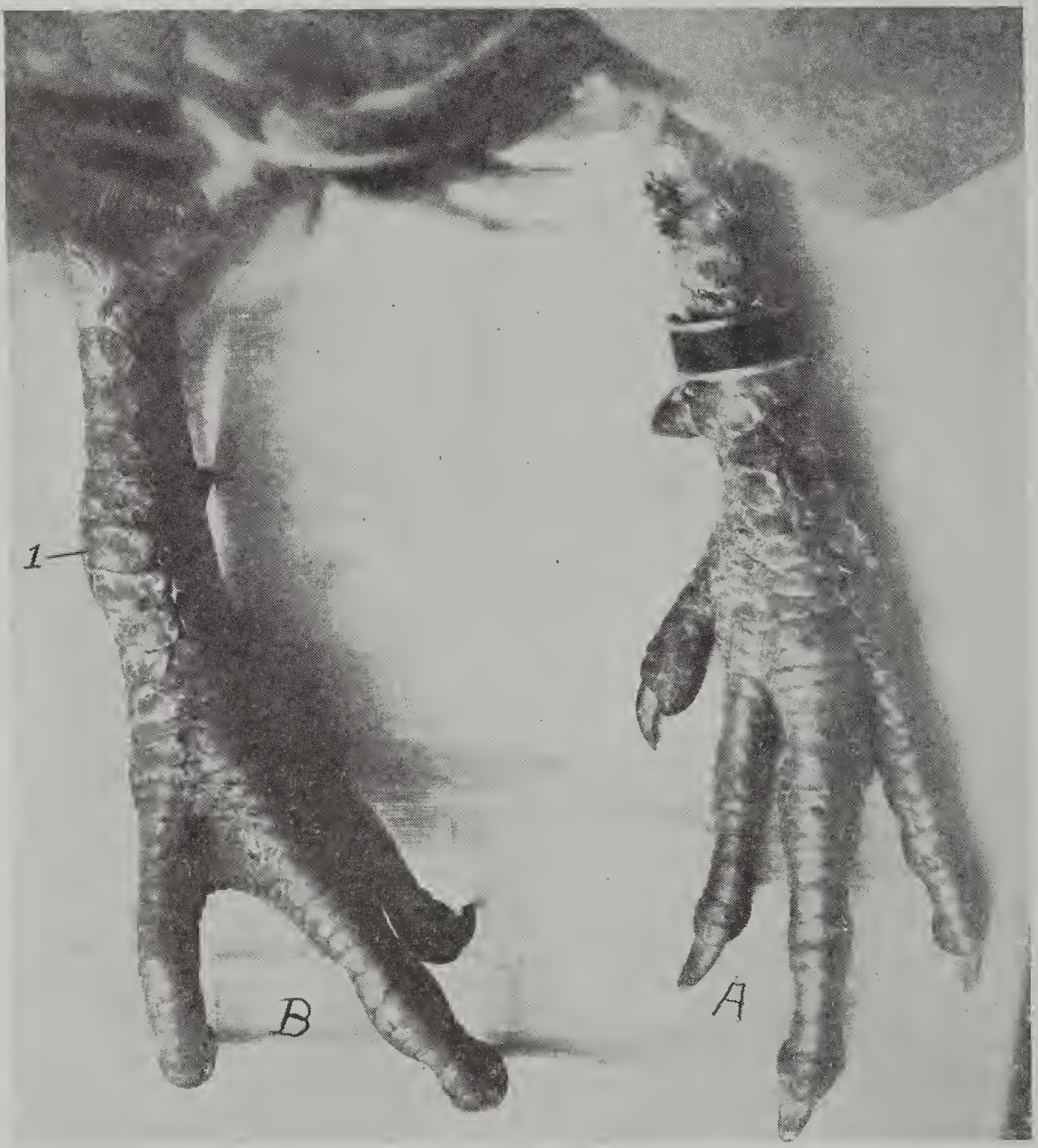

Figure No. 14

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TECHNICAL BULLETIN 15

A MONOGRAPH ON TREMBLES OR MILKSICKNESS AND WHITE SNAKEROOT

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AND B. F. KAUPP

NORTH CAROLINA
AGRICULTURAL EXPERIMENT STATION
CONDUCTED JOINTLY BY THE
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AGRICULTURE AND ENGINEERING

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CONTENTS

PAGE
Introduction 5
Historical Account of the Early Occurrence of Trembles and Milksickness.. 6
The Cause of Trembles and Milksickness 9
  a. Mineral Poison Theory 9
  b. Microbic Theory 12
  c. Poisonous Plant Theory 15
Distribution and Description of White Snakeroot 17
Comparison of White Snakeroot with Related Species 19
Names of the Disease 19
Clinical History 20

  Symptoms:

    a. In Cattle 20
    b. In Sheep 22
    c. In Hogs 23
    d. In Guinea Pigs 24
    e. In Man 24
  Mortality in Milksickness 25
Experimental Procedure 26
Results of Experiments 28
  Feeding Experiments with White Snakeroot 28
    a. With Sheep 28
    b. With Hogs 34
    c. With Guinea Pigs 35
  Transmission of the Disease 38
    a. By Milk and Milk Products 38
      1. To Lambs 38
      2. To Mice 40
    b. Through the Agency of Meat 40
      1. To Dogs 40
  Summary of Feeding Experiments 41
Post-Mortem Examinations 41
  Clinical Data on Cases 43
    Temperature, Pulse, and Respiration 43, 45, 49, 52, 55, 58, 60
    Clinical Notes 44, 46, 50, 52, 56, 59, 60
    Blood Study 44, 47, 50, 53, 56, 61
    Urine Study 47, 51, 53, 56, 63
    Protocol 44, 48, 51, 54, 57, 59, 61, 63
    Microscopical Examination 45, 48, 52, 54, 58, 60, 62, 63
    Cultural Examination 63
  Summary of Examinations of Sheep and Lambs 64
Nature of the Poisonous Principle 65
Means of Prevention and Cure 67
General Summary 68
Literature Cited 70
Illustrations 75

A MONOGRAPH ON TREMBLES, OR MILKSICKNESS, AND WHITE SNAKEROOT.*

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Introduction

For a number of years, complaints have come to this Station through letters from farmers, county agents, and extension workers, of the occurrence within the mountainous sections of North Carolina of a peculiar malady known as Trembles. This disease annually causes very considerable losses of horses, cattle, sheep, and hogs. Furthermore, in sections where this disorder is present, the belief is unanimously and firmly held that man may contract the disease by the ingestion of milk, hence the name "milksickness" which physicians employ, of certain milk products and of flesh of animals affected with trembles.

A questionnaire was therefore sent out to learn, among other things, something of the distribution of trembles, the seasonal incidence of the disease, the kinds of animals affected, preventive and curative measures employed, losses, and opinions as to the cause. It was found from these reports that the disease very probably occurs in the mountainous portions of all the counties in western North Carolina and in the immediately adjacent sections of States having similar conditions. The losses, furthermore, to the live-stock interests are so grave, since the danger of an outbreak is ever present and since so few affected animals recover, except when cases are discovered and treated in the incipient stages, that the farmer is reluctant to make any considerable investment in stock in sections where the malady is likely to occur.

Not only is an additional risk placed upon live-stock production by the presence of this disease, but it also discourages certain industries such as creameries and cheese factories whose success depends upon a lively interest in the growing of domestic animals. The popular attitude toward the disease is typified by the report of one county agent, who asserts that in his section trembles is the limiting factor in the establishment of cheese factories.

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Numerous beliefs, too, many of which are shrouded in mystery, were found to be entertained in regard to the causation of the disease. An examination of the voluminous literature on trembles and milksickness revealed the fact that many of these beliefs had been expressed in print, and that even critical investigations dealing with the etiology of the malady contained diverse conclusions. The one apparently most widely accepted by investigators, however, is that the disease is of bacterial origin. This opinion is contained in a recent bacteriological text-book* which states that the disease "is caused by the growth of and consequent absorption of toxic principles from the infective agent (*Bacillus lactimorbi*) lodged primarily in the small intestine and, secondarily, in the liver and other organs of the body."

In view, therefore, of the immense economic importance of trembles and milksickness and of the conflicting statements in regard to its etiology, a study of the disease was begun in the summer of 1916. This work was continued throughout the succeeding year and a considerable body of data has been secured bearing on the cause, transmission, symptoms, and pathological anatomy of the disease. A preliminary report on the cause of trembles has been published,† but since the subject-matter of this report is included in the present paper, further mention of it is deferred at this place. It is deemed advisable, furthermore, since the readers of this bulletin may not have ready access to published accounts of milksickness, and consequently may not be familiar with the literature bearing on the disease, to include a considerable body of historical information.

HISTORICAL ACCOUNT OF THE OCCURRENCE OF TREMBLES AND MILKSICKNESS

This disease has for a long time been known to occur within the United States. Attention was first directed to its presence by a physician, Dr. Daniel Drake, who published, in 1810, a brief account of it under the title "New Disease" in his Notices Concerning Cincinnati. This account, which was communicated to Dr. Drake, was based upon observations made in Madriver County, Ohio, by Dr. Barbee. Drake's account consisted of a concise statement of the symptoms, kinds of animals affected, conditions favoring its prevalence, and remedial and palliative measures. This account was later reprinted by Drake as an editorial addition to an article by McCall (1830). While this note in 1810 is the first published account, the disease is known with cer-

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*Vide, Marshall, Charles E., edit. *Microbiology,* a text-book of micro-organisms, general and applied. 2d edition, p. 785, 1917.

†Journal of Agricultural Research. June, 1917.

tainty to have existed prior to that time. Drake (1836) makes the statement "that this malady has been observed in some parts of North Carolina more than sixty years ago (*i. e.,* prior to 1776) and that it had been an endemic in certain localities of Tennessee, Kentucky, and Ohio from their first settlement." Coleman (1822) states that "sick stomach has been prevalent in some of the western counties of the State of Ohio since their first settlement." McCall (1823) makes specific mention of several cases which occurred in North Carolina between 1779 and 1800.

Rawlings (1874) also makes reference to "its first avowed recognition in the State of North Carolina—now over ninety-six years ago" (*i. e.,* prior to 1778). It is further stated in this account that "the disease followed emigration in its westward march from North Carolina to Tennessee and Kentucky, and prevailed in various parts of Ohio, Indiana, and Illinois. The report of Carney (1847) mentions the occurrence of the disease, "I think sixty or seventy years ago (1777 or 1787) in the Carolinas" and "upwards of fifty years since, it prevailed in Kentucky." In speaking of the disease in Tennessee, Lea (1821) asserts that "as soon as settlements commenced in the county of Franklin, about 12 or 15 years since, near the mountain, many cattle were lost from some unknown poison, the nature of which is still a mystery among the inhabitants. Occasionally whole herds have been found dead in some sequestered cove of the mountains." There are also early records of the disease in Indiana made by Faux (1823) and in northwestern Missouri by Long (1823).

An interesting historical account comes from the pen of Flagg (1836) from observations made while he was in Monroe County, Illinois. This states that "a mysterious disease called the *milk sickness*—because it was supposed to be communicated by that liquid—was once alarmingly prevalent in certain isolated districts of Illinois. Whole villages were depopulated; and though the mystery was often and thoroughly investigated, the cause of the disease was never discovered. By some it was ascribed to the milk or to the flesh of cows feeding upon a certain unknown poisonous plant found only in certain districts, by others to certain springs of water, or to the exhalations of certain marshes. The mystery attending its operations and its terrible fatality at one period created a panic in the settlers; nor was this at all wonderful. The disease appears now to be vanishing."

Other writers share this idea that the malady is disappearing since Winans (1840) states that "I had three to five hundred patients during the whole time the disease prevailed. It has now nearly or quite vanished away." Beach (1883) in speaking of the disease in Madison

County, Ohio, "presumes nearly one-fourth of the pioneers and early settlers died of this disease." Compton (1881) in discussing conditions in Dubois County, Indiana, in 1815, says that "more than half the deaths that occurred in that section were from milksickness. It was also very fatal among stock."

While these opinions represent the belief of a considerable number who have known this disease, it is by no means universally shared. Among those who have expressed themselves differently may be cited Smith (1874) of Kenton, Ohio, who states in a letter to a medical journal, "We are having, this fall, a regular epidemic of what is generally called milksickness or trembles. I have treated upwards of thirty cases." In an article by Gray (1881) twelve cases of so-called gastritis developed among the consumers of butter from a certain farm. Three of these came under his observation and were apparently genuine cases of milksickness. Shapard (1892), of Winchester, Tenn., believed that the disease was as prevalent as at any time within the past fifty years. As recently as 1907, McCoy (1907) investigated an epidemic which resulted in six deaths in Macon County, Tenn. A recent report by Selby (1917) from the Ohio Station records the death from trembles of a cow at Sharpsburg, Ohio, in 1912, and of two at Johnstown, Ohio, in 1915. A communication was furthermore received at the Ohio Station in 1916 concerning the death of a young woman near Cygnet, Ohio, from milksickness.

Examinations of records of this disease in the various medical journals indicate that milksickness was more prevalent during the first half of the nineteenth century than during the latter half. This is to be expected since the lands in which white snakeroot (Eupatorium urticaefolium) grows have gradually been cleared and brought under cultivation. Even those who did not recognize this weed as the etiological factor in the production of this malady unanimously agree in the observation that thorough clearing and cultivation of the land will transform a dangerous area into a safe one (Drake, 1849; Crook, 1897; Pickard, 1857; Way, 1893).

Several reasons may be brought forward to show that the relative prevalence of this disease in times past cannot now be determined with any degree of exactness. In the first place, the International Classification of Diseases and Causes of Death does not recognize the existence of milksickness. This is not surprising, when attention is directed to the fact that at the time when the disease was most prevalent some writers, who expressed themselves voluminously on milk sickness, were very skeptical as to the existence of any such disease. Among these are Yandell (1852), who in criticising the various publications dealing with the cause of the disease, gives it as his opinion that "the various accounts of this disorder nullify each other; and the mind is left in extreme doubt whether there is anything specific in milksickness." In discussing the existence of milksickness, Anderson (1867) has asserted that it is "a mere matter of credulous fancy." Other writers, as Chapman (McCall, 1823), Thompson (1854), and Bowen (Bailey, 1888) only go so far as to declare their disbelief in its existence.

A second reason for failure to determine the relative prevalence of this disease formerly is that health authorities have refused to record deaths reported under the name of milksickness. Again, genuine cases of milksickness have no doubt been diagnosed as gastritis, typhoid fever, ptomain poisoning, etc. This is to be expected since physicians have not had occasion to observe the malady while receiving their medical training, and so do not always identify it in practice.

THE CAUSE OF TREMBLES AND MILKSICKNESS.

The etiology of this disorder has been the subject of considerable speculation and investigation, but no one appears to have presented any convincing body of data in support of his conclusions. The solution of this question was regarded as of so much importance in the early part of the past century that rewards were offered by several State legislatures for the discovery of the cause. Even as late as 1904, Osler (1904) in addressing medical officers of the United States Army calls attention to this as one of the intricate unsolved problems.

Numerous articles have been written on the causation of this disease. In a general way, these can be classified as supporting one of the three following theories: (a) mineral poison theory, (b) germ theory, and (c) poisonous plant theory. One finds advocates also of other more or less plausible hypotheses some of which are mentioned later in this report. It is believed that the writers' evidence can best be presented after brief consideration has been given to some of the more important published accounts on this phase of the problem.

a. Mineral Poison Theory.

A number of observers, among whom are Shelton (1836) in Alabama, White (1836) in Indiana, and McAnelly (1836) in Kentucky, held that animals while grazing obtained some poisonous mineral which was present in the soil in affected regions. The last of these men believed also that this poison was present in well water and accounted for the occurrence of the disease in autumn by the lowering of the water table resulting in the poison becoming "more concentrated."

Among the minerals held to be the cause of milksickness are arsenic, copper, mercury, cobalt, lead, and aluminium. Seaton (1842) was a very vigorous supporter of the view that arsenic was the cause of the disease. His arguments were as vigorously opposed by Drake (1842) and by Yandell (1852).

McCoy (1907) failed to produce in guinea pigs any condition remotely resembling trembles in feeding experiments in which cobalt, lead, and arsenic were employed.

Moseley (1909) from feeding experiments with rabbits came to the conclusion that trembles was due to aluminium phosphate and that animals get this substance by eating white snakeroot. Proof of this claim consisted in feeding aluminium, which was present in the ash of white snakeroot and in the milk and butter of affected cows, and in developing trembles by the feeding of aluminium phosphate. He found this substance also in the ash of Bigelovia (rayless goldenrod) sent from New Mexico, where ranchmen claimed that it caused trembles among their cattle. In his experiments similar effects followed the feeding of rayless goldenrod, white snakeroot, and aluminium phosphate. Another paper published by Moseley (1910) about a year later affirms that soda exerts an antidotal effect upon aluminium phosphate.

One of the most potent reasons urged that the disease is associated with the soil itself, is a fact quite universally agreed upon by all observers. Cattle pastured upon particular tracts of land acquire milksickness, whereas others on adjoining pastures never show any sign of the disease. These milksick areas are believed to be so sharply delimited that farmers have fenced off areas to prevent the access of stock. In some places the changing of the fence so as to include even a few yards of uncleared land is held to be responsible for an outbreak of trembles. In White County, Tenn., McCoy (1907) states that the disease has been very rare in the locality of Milksick Mountain since it was fenced about sixty years ago. These enclosed areas sometimes occupy only a few square rods. Shelton (1836) records the fact that "in Blount County, Tenn., there is a locality embracing not more than ten or fifteen acres on which the disease has been known to originate for nearly, perhaps quite, forty years."

Wooded land has been held by a considerable number of observers to be essential for the existence of the disease. Drake (1841) from observations made in Ohio says "trembles in cattle, horses, sheep, and hogs are produced by their frequenting the densely timbered table land which from its flatness abounds in wet places and ponds, indicated by the presence of lofty white elms, black walnuts, maples, burr oaks, and other trees which delight in a rich moist soil." It is furthermore claimed by Lea (1821) and Beach (1884) that cattle may contract trembles if left overnight in such areas, but if they are removed before dark the disease can be avoided. When such wooded land has been cleared, it is rendered harmless. Crook (1857) claims that "in no instance has it ever been known in this county (Spencer County, Ind.) during any other than the grazing season, and then *only* upon lands that had never been in cultivation." This is in agreement with the claims made by Bennett (1822), Pickard (1857), and Simon (1888).

Attempts have been made, also, to associate the disease with certain soil types. Seaton (1841) claimed that it occurred only where sandstone entered largely into the composition of the soil. Those who did not agree with this view based their opinion upon the wide geographical distribution of milksickness with the consequent wide variety of geological peculiarities.

Another observer, Allen (1878), who believed that the disease is closely associated with the soil, records the peculiar instance that in certain localities in Effingham County, Ill., cattle lick large holes in the earth, after which many of them die.

In a considerable number of instances, water from infected areas has been held to be responsible for the disease. White, as early as 1836, mentions instances of outbreaks among cattle and sheep attributed to water. Beach (1883) gives an account of trembles in cattle contracted after having eaten hay from a meadow which had been flooded by surface drainage from a tract of land notorious for producing the disease. More recently Lyday (1896) maintains that in North Carolina the drinking of water from streams that flow from milksick coves is responsible for the disease. Fulton (1884) cites an instance in Logan County, Ohio, where cattle had taken the disease until a certain spring was fenced off, whereupon there was no more trouble. Credible informants told him of the disease in cattle accustomed to be watered at a certain well in Clark County, Ohio. When the well was closed, the disorder disappeared. He also recounts an instance of "a farm in Hardin County on which members of every family that has ever lived there have had the disease. On that farm I have attended patients who said they had not used meat, milk, butter, or cheese for weeks, guarding against the disease. The presumption, therefore, was that they and also those of previous families that had lived there had obtained it directly from the water."

Others have considered water responsible for the transmission, and thus indirectly allign themselves with the germ theory to be next discussed. McAnelly (1836) states in support of water as the agency of transmission, that "the disease sometimes prevails in the human species in districts where the inferior animals are comparatively exempt and frequently where none of them are known to be affected at that time." The following singular statements by Brewington (1876) express belief in the same idea: "I do not believe that in this vicinity there is one in fifty persons that take the disease who gets it by using milk or butter from diseased animals. In many places where the disease used to be most prevalent, the people used water from wells for cooking and drinking, but since they built cisterns and use water from them exclusively, they are entirely exempt from the disease."

b. Microbic Theory

Several early students of milksickness doubted the validity of the mineral poison theory as an explanation of the causation of the disease and manifested a belief in the microbe theory. They did not have the advantages, however, of modern bacteriological technic and, basing their judgment largely on the transmissibility of the disease, concluded that it must be caused by some microscopic organism. Both Wright (1827) and Reed (1856) felt that the failure of chemical studies to show the presence of a mineral poison and of botanical studies to inculpate any poisonous plant indicated that the cause must be sought among cryptogamic parasites.

Because of the transmission of the disease through meat and milk, Burger (1825) compared milksickness with anthrax. Heeringen in 1843, twelve years before the discovery of the anthrax bacillus, and Heusinger in 1853 also pointed out certain points of similarity between milksickness and anthrax. Wood (1858) on purely *a priori* grounds argued causation by a germ. The passage of the disease from one body to another and "its multiplication in the system" inclined Byford (1855) and DeBruler (1858) to the germ theory. Philips (1877) found "a great number of living, moving, spiral bacteria" in the freshly drawn blood and in the urine of a typical case. Woodfin (1878), too, believed that milksickness is a specific infection. Gardner (1880) reported finding "countless multitudes of actively moving, writhing, twisting bacteria" in the blood of a heifer suffering from trembles and in dogs affected by eating the flesh of this heifer. He furthermore found the same organism in the blood of two human patients, in the water of a spring which supplied this stricken family, and in milk. Microscopic examinations of blood by Schmidt (1877) failed to reveal the presence of bacteria.

Logan (1881) committed himself to the statement that the disease must be due to a "contagium vivum."

It is a common belief in some sections of the State that cattle exposed at night in "milksick areas" contract the disease. This has suggested conveyance of the infection through the bites of arthropods or certain insects.

A considerable number of writers who have studied the disease more recently than the ones just reported accept the theory of a parasitic origin of the disease. Among them are Maurel (1884), Boggs (1907), McCoy (1907), Crawford (1908), Jordan and Harris (1908, 1909), and Luckhardt (1909). The three last named authors alone have reported studies upon a definite microscopic organism. Jordan and Harris (1908, 1909), in their two extensive accounts, record the finding of an undescribed spore-forming bacterium, which they designate as Bacillus lactimorbi, in the liver, bile, and spleen of a heifer, in the liver of her fetal calf, in the pericardial fluid and gut nodule of a horse, in the brain, heart blood, and liver of lambs, in the feces of nonfatal cases in man, in the milk of cows, in the soil of milksick regions and in regions where milksickness has never been known, in normal cow dung, and on various grain and forage plants. They succeeded in several instances in isolating B. lactimorbi in pure culture from the heart blood and from certain internal organs of animals examined a few hours after death. They used in their experimental work rabbits, guinea pigs, dogs, cats, lambs, and calves. It may be briefly stated with reference to this work, that no well marked cases resulting in death were developed, although certain symptoms and lesions similar to those in animals naturally affected with milksickness were produced. In concluding their report, they assert that "taken as a whole, the facts do not indicate that a specific microorganism is the cause of milksickness or trembles. The bacillus in question belongs to a group which seems widely distributed and for the most part certainly not endowed with pathogenic qualities."

Luckhardt (1909) made further studies in the laboratories of Drs. Jordan and Harris on the physiology of Bacillus lactimorbi, on its distribution, and on its pathogenicity. He isolated the organism from alfalfa from farms in Wisconsin, Illinois, and Indiana, which supplied certified milk to Chicago and Milwaukee, and from four species of weeds, Bigelovia Rusbyi, Solanum elæagnifolium, Gutierrizia sphærocephala and Portulaca pilosa from the Pecos Valley, New Mexico. He furthermore isolated it twice from Roquefort cheese. Dogs were used in inoculation experiments. In discussing the results of this work the author concludes that "the preceding experiments are far from being decisive in establishing B. lactimorbi as the etiological factor in the production of milksickness. The organism either loses its pathogenicity very rapidly when grown on artificial media, or the virulence of the organism is dependent upon what may be termed a symbiotic life or existence on certain plants. . . . On the other hand, it is remarkable that if B. lactimorbi be the cause of milksickness, it should have so wide a distribution in milksick and nonmilksick regions."

In a few cases certain molds and mushrooms have been claimed to be etiological factors. Among those who suggest that mushrooms are the poisonous agent are Winans (1840), Johnson (1866), and Gardner (1880). Hessler (1905) reports the finding of Sterigmatocystis, one of the cosmopolitan molds, in the blood of an affected heifer. Slack (1854) compared milksickness to ergot poisoning and believed the cause to be a "fungus production" on grass or grass seeds, a view also held by Nagle (1859).

Several other more or less fanciful causes of milksickness which cannot be classified under either of the three groups of causes have advocates within North Carolina and will, therefore, be briefly treated before giving consideration to the poisonous plant theory. Walker (1886) experimentally exposed corn fodder to the dew in a milksick cove and thus communicated trembles to a yearling. Farmers in some sections of North Carolina have come to believe that cattle which are penned up at night and not permitted to graze before the dew has evaporated are not liable to contract the disease. This same opinion has been expressed in the writings of Evans (1860), Reagan (1884), and Walker (1886). The influence of dew is only an apparent one as explained, in part at least, by Woodfin (1878), who says that cattle which are confined at night cannot range sufficiently far to reach the affected and usually remote tracts of land. The influence of dew is explained by Davis (1881) on the ground that animals eat more greedily of plants wet with dew than when they are dry.

A number of writers have adhered to the idea that the disease is produced by a gas or miasm rising from the earth. Lea (1821), Horne (1846), Jones (1862), and Wozencraft (1873) employ such terms as "an exhalation from the soil" and "peculiar miasma" in accounting for the disorder.

The claim has furthermore been made by Lescher (1850), Thompson (1853), Rawlings (1874), Dorsey (1876), and Sale (1871) that the disease resembles, in some respects, malaria.

Two accounts, Kennedy (1878) and Achelor (1884), which have come to hand, attribute the disease to the bites of a certain fly, an idea which would liken milksickness to the tsetse-fly disease or sleeping sickness in Africa. This idea is vigorously opposed, however, by the writings of Carpender (1884) and Murfin (1884).

c. Poisonous Plant Theory

Many physicians and laymen have held to the theory that trembles is caused by the ingestion of a poisonous plant. One reason for this view is the general accord of observations to the effect that "milk sick" tracts are no longer able to communicate the disease after the natural vegetation has been replaced by cultivated plants. Bennett (1822) is among the earliest writers to record this fact. He states that "those farmers who keep their cattle and horses in enclosed pastures which have been cultivated do not lose them by this disease." This opinion is also in accord with the writings of Crook (1857) and Simon (1888). Pickard (1857) cites an interesting example which substantiates the view that clearing and cultivation are efficacious in destroying the dangerous quality of milksick lands. He reports that a "family having suffered from its ravages, plowed up a pasture field, digged around the stumps, thoroughly turning all the soil, and sowed the field in grass, upon which they have kept their stock for twenty years, and at no time has milksickness made its appearance; while on other portions of the farm, uncultivated, it is as fatal as ever." The same opinion is expressed by Drake (1841), who records that "clearing and cultivation, even girdling the trees, harrowing the ground, and sowing it with grass seed destroys and renders inactive the cause, whatever it may be."

Another reason for belief in poisonous plants as the cause of trembles, is that the disease is largely limited to the season when animals are allowed to graze. That the disease may occasionally appear in winter is attested by the writings of Yandell (1852), Beck (1857), Waggoner (1859), Beach (1884), and Shapard (1892). This may be accounted for by the feeding of hay as has been recorded by Gray (1881), Beach (1883), and others. The latter makes the following interesting statement: "That cattle *may* have trembles in the winter season is a matter of occasional observation, and the old citizens generally attribute it to the feeding of cattle upon wild or swamp hay." Beck (1857) has stated that in Kentucky the disease is prevalent only in winter, whereas, in Indiana it occurs in all of the other seasons.

It seems to be the common belief among farmers in North Carolina that the usual time of prevalence is during the months of August, September, October, and November. Dawson (1842), Rawlings (1874), and Shapard (1892) refer to the greater prevalence of trembles in late summer and autumn. Dewey (1845) speaks of its disappearance after the first hard frost. While the autumnal incidence has been found to be most frequent, it may also occur during spring and early summer. An epidemic in Tennessee recorded by McCoy (1907), to which reference has previously been made, occurred during April and May. In the light of evidence to be presented later in this paper, there is no reason for believing that the disease may be limited to any one season. If it chances to be more prevalent in late summer and fall, as is commonly believed in this State, it can be accounted for by the refusal of animals to eat of the weed until this season of the year. A scarcity of more palatable plants through drought may compel them to eat more freely at this season of white snakeroot than is their custom.

A considerable number of flowering plants have at various times been held to be the cause of trembles. Among them are the following: Rhus toxicodendron (poison ivy), Eupatorium urticaefolium (white snakeroot), Lobelia inflata (Indian tobacco), Cicuta maculata (water hemlock), Bigelovia Rusbyi (rayless goldenrod), Psedera quinquefolia (Virginia creeper), Symphoricarpos orbiculatus (coral berry), Apocynum cannabinum (Indian hemp), Caltha palustris (marsh marigold), Euphorbia esula (leafy spurge), and Aethusa cynapium (fool's parsley). Through the writings of Drake (1836 and 1841), Travis (1840), Crook (1857), Chase (1861), and Elder (1874), poison ivy was at one time quite generally accredited as being the cause of the disorder. Henry (1854) and Beach (1883) pointed out that horses, cattle, and sheep frequently eat the foliage of this plant with manifestly no ill effects. The connection of poison ivy with trembles is further opposed by Landrum (1861) and H. M. K—— (1862), who assert that milksickness has never occurred in certain localities where poison ivy grows luxuriantly.

Claims that a number of other plants are the probable cause may be found in the accounts of McCall (1822), Short (1840), Fisher and Kennicott (1861), and Allen (1878), but these authors adduce little evidence in support of their conclusions.

White snakeroot has probably been more commonly accused of being responsible for trembles than any other plant. As long ago as 1840 Dr. Barbee, who will be recalled as having first given information to Dr. Drake which led to the publication of the first account of the disease, expressed the opinion that this weed was the cause of the disease. A few years later Dewey (1854) stated that the disease is due to "a succulent plant bearing a white blossom." He did not know the name of the plant and failed to have it identified, but it is very probable that he had reference to white snakeroot. Subsequently, evidence adduced by feeding experiments conducted by Vermilya (1858), Jerry (1867), Townshend (1883), and Moseley (1906) point to white snakeroot as the etiological factor in the production of milksickness.

Moseley's account, beyond doubt, contains the best experimental evidence which had been presented up to that time on the poisonous properties of this weed. His results were not entirely convincing, however, since Crawford (1908), in criticising them, states: "It cannot be said that Moseley has even proved Eupatorium urticæfolium to be a poisonous plant, much less the cause of "trembles." Beach (1883) had previously recorded the observation that cattle frequently eat this weed without any apparent ill effects.

The most extensive recent studies, except those published in a preliminary report from this Station (1917), bearing on the toxicity of white snakeroot are those of Crawford (1908), which were undertaken because of an outbreak of trembles resulting in the death of about 50 head of cattle near Minooka, Ill. Patches of white snakeroot which had been browsed were found in pastures where the animals had contracted the disease. Some of these weeds were collected and dried, while others were preserved in water to which a small quantity of chloroform had been added. Aqueous extracts were then prepared and fed or injected subcutaneously into rabbits, cats, and dogs No cases of trembles developed in these animals and Crawford became so convinced of the innocuous nature of the plant that he took without ill effect a water extract from 400 grams of fresh Eupatorium urticæfolium. These results with aqueous extracts are in accord with those secured by the present writers, and which are discussed later in this report.

Crawford furthermore reports that he fed 58 grams (about one-eighth pound) of fresh plant to a lamb weighing 25 kilograms (about 55 pounds) with the production merely of some diarrheal symptoms. In criticising this experiment, Moseley (1909) says that this quantity would probably not be fatal to a full grown rabbit. In his summary of these experiments, Crawford states "It cannot be said that it has been proved that milksickness is due to any constituent of Eupatorium urticæfolium."

The preliminary account referred to above contains the results of feeding white snakeroot to sheep during the season of 1916. Since the present season's work has been in part a confirmation of that of 1916, it is deemed best to combine all of the data bearing on any phase of the problem. These data will, therefore, appropriately appear later in this report.

DISTRIBUTION AND DESCRIPTION OF WHITE SNAKEROOT.

White snakeroot is a member of the composite family and belongs to a genus which is represented in the southern United States by about forty species. The plant is also known by the common names white sanicle and rich weed, and bears the technical name Eupatorium urticæfolium Reich., which is synonymous with E. ageratoides L. f. and E. boreale Greene. The specific name indicates that the leaves resemble in appearance those of the wood nettle. This plant (Plate 1) grows in rich woods bordering streams, being confined to rather low, swampy situations, or within shaded mountain coves, particularly on northern slopes. It often grows so luxuriantly in these latter situations within North Carolina as to become the principal vegetation on the forest floor.

It is evidently very widely distributed since its range is said to extend from New Brunswick southward to Florida, Georgia, and Louisiana, and westward to Nebraska and Oklahoma.

Specimens in the herbarium of the New York Botanical Garden* show that it has been collected in Ontario, Maine, Vermont, Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, District of Columbia, Virginia, West Virginia, Georgia, Louisiana, Michigan, Illinois, Indiana, Minnesota, Iowa, Nebraska, Oklahoma, and Kentucky. This would indicate that it very probably occurs in all of the other States east of the Mississippi river as could best be ascertained by examinations of the Gray Herbarium at Harvard University and of the United States National Museum.

The plant is perennial rooted, varies in height from one to five feet, and is more or less branched. The leaves are three to six inches long, opposite, long stalked, and thin. They are ovate or slightly cordate in outline, are pointed, and the leaf margin is coarsely and sharply serrate. The base of the leaf is cordate or is abruptly narrowed into a long, slender petiole or stalk. Furthermore, the leaves are strongly three-ribbed. The flowers open from July to November, and are very attractive since they are clustered in snowy white, compound corymbs. The involucre is narrowly bell-shaped or campanulate, being composed of linear, acute bracts. The achenes or seeds are smooth.

The plants are for the most part smooth, but some possess a coating of hairs. To those which are distinctly hairy, the appropriate varietal name villicaule is applied by some. Intergrading forms between the smooth and hairy forms may be found in any area where this plant occurs.

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*This information was furnished through the kindness of Mr. Percy Wilson of the New York Botanical Garden, to whom thanks are hereby extended.

COMPARISON OF WHITE SNAKEROOT WITH RELATED SPECIES

Some of the species of Eupatorium are distinguished with difficulty from white snakeroot, which accounts for the fact that farmers on being shown white snakeroot and told that it is the cause of trembles, not infrequently assert that their stock feed upon the plant without any ill effects. The casual observer cannot depend upon the color of the flowers to distinguish species, since more than thirty species of Eupatorium have white flowers. Furthermore, Eupatorium incarnatum, which may have flowers tinged with pink or purple although frequently they are white, is practically indistinguishable from white snakeroot except by the presence of minute hairs on the corolla tube. These can be seen only with the aid of a hand lens.

Twenty-nine species with opposite leaves have been described. Only seven of these, however, including white snakeroot, have manifestly stalked or petioled leaves. In this group five possess leaf blades which are similar in shape to those of white snakeroot. The leaf blades of the other one are fully twice as long as broad, which makes its separation easily possible.

Whether or not the roots are perennial is a distinguishing character, since only five species with perennial roots, in which group white snakeroot is included, have been listed. Two of these are shrubby and the other three are herbaceous. In the latter group, the character of the leaf margin, whether it be provided with sharp or blunt teeth, is used in separating E. urticæfolium from E. aromaticum, and the absence of a beard on the corollas separates these two species from the other, E. incarnatum.

The direction of attention to these points of similarity between white snakeroot and other species of Eupatorium will, it is hoped, serve to indicate that careful observation and experience are necessary in determining whether one is dealing with white snakeroot or some of the other species which are not known to be poisonous, but which are commonly present within the State.

NAMES OF THE DISEASE

This disease has been appropriately designated as trembles in animals, because trembling is the most obvious symptom, and as milk-sickness in man, because it may be communicated to man through milk from cows affected with trembles. It has been given a number of other names, but it is not possible because of the mystery which has for so long surrounded the cause of the malady to determine whether, in every instance, reference is made to the same disorder.

Since obstinate vomiting is such a prominent symptom of the disease in man, physicians and others have very commonly employed the name sick stomach, Beck (1822), Bennett (1822), Campbell (1881), Chamberlain (1826), Crookshank (1826), Drake (1836), Drake and Yandell (1840, 1841, 1842), Haines (1822), McCall (1830), Wright and Bennett (1827), and Yandell (1832).

The name "tires and slows," employed by Howard (1871), Logan (1849), and Byford (1855) are used in some sections. Jordan and Harris (1909) are of the opinion that "alkali poisoning" of western Texas and New Mexico is identical with trembles or milksickness in States east of the Mississippi River. Other names, which have at one time or another been employed but which have not been generally used, are swampsickness, river fever, puking fever, stiff-joints, colica trementia, morbeo lacteo, ergodeleteria, gastritis, gastro-enteritis, mukosoma, syro, lacemesis, caconemia, and paralysis intestinalis.

Clinical History

Symptoms

The published reports of observations by others are essentially unanimous in stating that horses and cattle are both very subject to the disease. Sheep and goats may furthermore be affected and a number of writers have asserted that hogs are susceptible. McCall (1822), Coleman (1822), Drake (1840), Winans (1840), and Davis (1881) are among those who claim that hogs are subject to the disease. Some writers have claimed that hogs are at most only rarely affected—Woodfin (1878) and Johnson (1866). It has been stated, too, that deer may suffer from the disease, and that other wild animals, such as wolves, foxes, buzzards, vultures, and crows may contract trembles by feeding upon carcasses of animals which have died of this disease.

a. In Cattle.—The symptoms of trembles among domestic animals have been described with no great degree of fullness except in cattle. The following account is substantially a concensus of all who have observed the malady. The first sign of the disease is indicated by a listlessness and disinclination to move. In his memoir, Drake (1841) has well characterized the initiation of trembles by stating that "the animal begins to mope and droop, to walk slower than its fellows, to falter in its gait." Practically all accounts record the exhibition at this stage of muscular weakness and of trembling, especially when the animal is driven. Such animals, too, are generally constipated. Some few writers record that cattle in this stage are greatly excited and are disposed to fight; this is not mentioned, however, by the great majority of observers. When the disease has progressed sufficiently so that the characteristic trembling ensues, there is a marked stiffness of the joints. The animal may sink to the ground manifesting great weakness and exhaustion, and may remain lying for hours or days on the spot where it has fallen. Animals in this stage may recover, but more often die.

One peculiar feature of the disease, *i. e.,* that violent exercise has much to do with the development of acute symptoms, has been generally reported and is in accord with statements by a considerable number of farmers within the State. Prospective buyers of cattle from "milksick" areas make use of this fact and drive the cattle until they are heated before deciding whether it is safe to purchase. Dixson (1833) early noted this and says "It is one of the peculiarities of the disease that the poison lies long dormant in the system, and only becomes active when excited by some indiscretion or violent bodily exertion." Woodfin (1873) states that "beeves have been fattened in these infected localities, started to be driven to market, take the trembles and fail the first or second day, while other cattle feeding on the same grounds, left at home, remain healthy."

This is entirely in accord with observations made by Mr. W. R. Radford and Mr. James Phillips in Mitchell County, at Spruce Pine, N. C., during the present year. Mr. Phillips purchased a herd of 20 steers and heifers which had pastured in Wiggins' Cove, on the farm of Mr. S. T. Henry, where white snakeroot grows profusely. The animals were driven to the shipping station, several miles distant, and upon arrival at the station five began to tremble. They remained lying down most of the time for several succeeding days, and when made to rise would stagger along for a short distance and then fall suddenly. They had all apparently recovered, however, after about a week.

The belief, which prevails in some sections, in the occurrence of the disease in the autumn only, may be accounted for in the light of this peculiar fact, if it is kept in mind that in these places it is the practice to drive cattle to market at this season of the year.

Another peculiarity which has been recorded by a number of observers is that the breath of affected animals has a foul odor. This has been described as "garlicky," "like chloroform linament," and "mildly like acetone." Elder (1874) speaks of it as a "singular fetid odor, pungent, and corrosive."

The fact that not all cattle in the herd become affected has always been a disturbing factor in accounting for the cause of trembles. As long ago as 1822, Coleman notes that "Cattle and horses do not always become afflicted with the disease if allowed to range in woods where the disease is common." Woodfin's (1878) comments bearing on this point, if judged in the light of experiments with sheep to be presented later, are equally true and interesting. He says: "Many animals may feed on the same lands and evidently partake about equally of the poison and some of them suffer violently and die; others suffer lightly and recover, and others still, perhaps the larger number, remain quite healthy."

b. In Sheep.—Very little that is distinctive can be gathered from the published accounts of the symptoms of the disease in other domestic animals. Writers have largely resorted to the use of the expression "similar to those of the disease in cattle" in describing the symptoms.

The only clinical account of the disease in sheep which has come to our attention is that given by Jordan and Harris (1909) from observations made by D. D. Todd upon two ten weeks' old lambs. Both of these animals were lying down when found, and one made no attempt to rise, while the other kept its feet going in a walking motion with each unsuccessful effort to get up. Respiration was regular in both animals but forced and jerky in the case of one lamb. No sign of pain was evident, but occasionally one animal ground its teeth.

In the present studies in sheep considerable individual variation has been found to exist, both in the period elapsing until the initial symptoms of trembles are apparent and in the period following until death ensues. During the season of 1916 some were sick as early as three days after being placed on the experiment, and no ill effects were apparent for three weeks in other cases. During the present season (1917) one animal became sick on the second day and died within forty-eight hours after feeding was begun. Others remained apparently normal for about twelve weeks before contracting the disease. Sheep usually live three or four days after the disorder is first noticed, but some have been observed to die within twelve hours. Only one sheep characteristically affected entirely recovered and the feeding of white snakeroot to this animal was discontinued as soon as trembling was noted.

One of the first signs of the onset of the disease is a loss of appetite and gritting of the teeth. Such animals are sluggish and manifest a marked disinclination to move. They may remain standing in a droopy posture (Fig. A) or may be quiet in a normal resting position. Respirations are accelerated, often jerky and somewhat labored. A marked stiffness of the legs and ataxia characterize the movements in walking. This is manifest early and becomes more aggravated as the disease progresses. If after a day or two, the animal is urged to rise and is driven a few yards, muscular spasm, especially in the limbs, is evident. The sheep then refuses to move, stands with hind limbs placed well under the body (Figs. A and B) and all feet spread apart laterally. In this posture, the back is bowed, the neck outstretched and the head lowered. Within a few seconds, the quivering spreads from the limbs over the entire body, increases in intensity, and becomes a violent, involitional tremor (Fig. B). This is accompanied by slight, intermittent, tetanic contractions of the musculature of the limbs. At this stage of trembling ataxia is very pronounced, and the animal is unable to stand (Fig. C). It drops quickly into the normal resting posture (Fig D) or assumes a characteristic position, in which the head and neck are outstretched and the jaw is resting upon the ground (Fig. E), whereupon the trembling apparently immediately ceases. If the sheep is made to rise after it has lain down for a few moments, a second and more violent spasm of trembling ensues with a repetition of the symptoms as described. Trembling may recur repeatedly every time the animal is made to rise. The quiescent period is shortened, however, after each spasm of trembling and may begin as soon as the animal is placed on its feet. Even after the disease has progressed to the point where trembling is violent, many animals still possess considerable appetite.

After the second or third day, the animal becomes comatose and may lie prostrate on its side (Fig. F) until death occurs. There is generally a very considerable decrease in weight, most of which occurs during the two or three days preceding death. The feces generally remain normal, with, in some cases, a small amount of mucus. Urination is more frequent than in healthy animals and only a small quantity is voided each time. Vomiting occurred in the case of two animals under observation, and a frothy discharge from the nostrils is not uncommon after coma sets in.

c. *In Hogs.*—The symptoms of trembles as observed in hogs are in general like those in sheep. There is first a listlessness, not accompanied, however, by any marked loss of appetite. The animal remains lying unless urged to rise, or unless food is offered, whereupon it stands with hind feet placed well under the body. In this position, the head droops, the eyes are partially closed, and the back is bowed. (Fig. I.) There is no lack of coordination of movement in walking for the first few days, although the hind legs are markedly stiff. Later, this stiffness appears in the fore limbs. After the pig has stood for a few minutes while eating at the trough or is driven around in the pen a slight quivering in the legs begins. Within a few moments, the trembling has become progressively more severe, has spread over the entire body and becomes a violent shaking or tremor, so acute that the animal squeals, presumably with pain, in its efforts to remain on its feet. At this stage of trembling the animal either falls to its knees or sits upon its haunches (Fig. J). From this position, after a moment, it falls into the resting position (shown in Fig. K), in which the feet are folded under the body and the jaw resting upon the ground, or it falls prostrate on its side with legs outstretched (Fig. L). Trembling continues for some little time after the animal has lain down.

Three or four days after trembling is first noted, it is not uncommon for the animal to topple from a standing posture to one flat upon its side. Coma appears in the 24 or 48 hours preceding dissolution.

d. In Guinea Pigs.—The disease is first apparent in guinea pigs by their refusal to eat. They are less active than unaffected animals and crouch in a corner of the cage. The hair upon the head and neck of sick animals does not lie down smoothly and their eyes remain only partially open. When such an animal is disturbed, it walks with the back arched. As the disease progresses, they become more listless, refuse all food, and become emaciated in consequence. Commonly diarrheal symptoms are present. Muscular tremors occur, but are not easily apparent since the animals cannot be made to stand nor to walk. Finally stupor sets in, at which stage they are very limp, with insufficient control of their limbs to enable them to rise when placed on their side. The half-closed eyes and general debility as indicated by the droop of the ear and posture are shown in a comatose animal in Fig. H.

e. In Man.—Several excellent descriptions of milksickness in man have appeared, in all of which the symptomatology appears to accord fairly uniformly. In the accounts of Coleman (1822), Graff (1841), Byford (1855), and Way (1893), attention is directed to the most typical symptom, "sick stomach."

The onset of the disease is gradual, and after a day or two of weakness and debility, accompanied by loss of appetite, the patient is seized with epigastric distress. Excessive vomiting follows, the bowels are obstinately constipated, and there is always great thirst, although the water taken is vomited immediately. Abdominal pain is generally present, although some writers record an absence of distress in this region, and muscular tremors are generally present. The breath acquires a peculiar foul odor, a characteristic which some regard as of great diagnostic value, since they maintain that it can be detected immediately upon entering the patient's room. The tongue is swollen. Respiration is normal and temperature is usually subnormal, ranging from 97 to 98 degrees F., although McCall (1822), Simon (1875) and a few others state that fever exists. If the disease is severe, symptoms of typhoid may appear. The patient may become delirious, but in fatal cases a period of coma precedes dissolution. In such cases, death may come as early as two days after the onset of symptoms or may be deferred for two or three weeks. Lasting debility appears to be a common sequel of the disease. Those who have recovered are usually incapacitated for hard work especially during warm weather. The writers themselves have never seen a person suffering from milksickness, but conversations and correspondence with doctors who have treated cases, and with persons who have recovered from an attack, indicates that the preceding account of symptoms describes the disease.

Mortality in Trembles or Milksickness

No data are available from which even an approximation of the fatalities among domestic animals can be made. One writer has estimated that 5,000 animals have died from the disease in a certain area in northern Ohio. The outbreak near Minooka, Ill., which occasioned the investigations of Crawford (1908), previously referred to, resulted in the death of about fifty cattle. Mr. H. E. Crawford, Shooting Creek, N. C., reports the loss of hundreds of dollars worth of horses, cattle, sheep, and hogs, and that in almost every case affected animals succumbed.† In a flock of sheep which came under the observation of Mr. George Evans, Sheep Field Specialist at this Station, seven animals in a flock of eight died. In our feeding experiments with sheep, only one individual characteristically affected recovered among a total of thirty-one animals. In the succeeding year this animal was again used in feeding experiments and contracted the disease and died. All accounts which have come to hand, both published and verbal, indicate that relatively few animals recover from a prolonged attack of trembles.

The data on the mortality in man from milksickness are by no means complete and considerable differences of opinion are found to exist in published reports. Coleman (1822) reports the loss of "about one case in twenty or thirty," and Collins (1902) "about 40 per cent of my cases." Dr. J. E. Wilson, Canton, N. C., informs us that he has treated many cases without the loss of one.

Jordan and Harris (1909) have prepared a tabulation of all published reports in which the number of cases and deaths is specifically stated. This tabulation will emphasize the variation which has been mentioned and will give at least an approximation of the average mortality. It will, therefore, be quoted *in toto,* and to it will be added the record of fifty-six cases and eleven deaths which either came under the observation of Jordan and Harris or were reported to them during their investigation of this disease, and of two deaths at Losantville, Indiana, in 1916.*

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†From a letter by Mr. E. S. Millsaps, County Agent, Clay County.
*From a letter by Dr. N. F. Canady, Hagerstown, Indiana.

Cases	*Deaths*	*Authority*
4	2	Lewis (1829)
10	5	Carney (1847)
1	0	McNutt (1857)
49	26	Sale (1871)
50	7	Smith (1867)
30	0	Smith (1874)
3	2	Houser (1880)
22	4	Spalding (1881)
3	1	Gray (1881)
4	2	Simon (1888)
11	6	Pusey (1880)
6	5	Graff (1841)
9	5	Tuller (1889)
2	0	Scott (1889)
6	1	Hurd (1875)
13	2	Sykes (1891)
6	1	Lyday (1896)
9	1	Mich. S. B. H. (1897)
5	2	Collins (1902)
13	6	Palmer (1904)
6	6	McCoy (1907)
56	11	Jordan and Harris (1909)
2	2	Canaday (1916)
320	77	

The mortality in man as judged by these data is seen to be approximately 24 per cent.

EXPERIMENTAL PROCEDURE

Since white snakeroot does not grow in the vicinity of Raleigh, N. C., where these feeding experiments were conducted, it was arranged to secure daily shipments of the green weed from Shooting Creek, N. C., where this plant grows luxuriantly. Since this town is over 400 miles distant from Raleigh, the weed used had been collected about 48 hours prior to its arrival at Raleigh. The weed was fed twice daily along with a maintenance ration of some dry concentrate. In the feeding experiments of the present season (1917) this feed was further supplemented by timothy hay. The sheep were kept singly in small pens in a sheep barn. A litter of shavings covered the concrete floors. The animals used were selected from a flock of grade ewes and their lambs, all of which were in a healthy condition, and, with the exception of those used in the experiments, remained so. No case of trembles or milksickness had ever appeared in this or any other of the Station flocks prior to the initiation of these experiments and none developed during the course of these experiments except in animals under experimentation. The flock number of each individual was retained and is used subsequently in reporting the experiments with the several animals.

At first the grain and weed were fed separately; but since the animals either avoided eating any of the weed or ate only sparingly of it, the weed was passed through an ensilage cutter and then mixed with grain before being fed. A daily account was kept of all of the feed which was refused by each animal, and these data were employed in approximating the total amount consumed during the course of the experiment. Since the weed and grain were mixed, and since some loss of weight was due to desiccation, the quantity of snakeroot eaten by each animal could only be approximated.

In the experiments with suckling lambs, the ewes and their lambs were kept separated except while the lambs took their milk. The ewes at these times were put into the pens with the lambs, so that the lambs never had access to white snakeroot, as would have been the case had the lambs been permitted in the ewes' pens.

Before feeding the weed to pigs, which were confined in pens in the sheep barn, it was passed through an ensilage cutter and mixed with cracked corn and shorts.

The dogs, which were fed upon the carcasses of sheep dead of trembles, were confined in pens and given, in addition, dog biscuits and refuse from the kitchen.

The guinea pigs which were used in these experiments had never been used in experimental work and were consequently entirely normal. During the experimentation they were kept in cages especially constructed for housing small animals, and were given a diet consisting of a mixture of cracked corn, wheat, oats, and rye, which was supplemented by green grass and clover. All animals used in any one of the experiments to be subsequently reported were kept together in the same cage. Each group of animals could thus conveniently be fed twice or three times daily with white snakeroot or some of the products prepared from this plant.

The material extracted by boiling the plant in water, in 2 to 3 per cent hydrochloric acid, or alcohol, for twenty-four to forty-eight hours, was evaporated to dryness on a water bath, ground into powder, and placed in No. 2 Lilly New Process Gelatine Capsules. The desiccation of these products necessitated heating for a long time because of the resinous nature of the extracted material.

The animals were held while feeding them these capsules, and were given from six to twelve capsules daily. By the exercise of considerable patience, the guinea pigs could be made to masticate and swallow the capsule and its contents. This method of administering the products of white snakeroot was abandoned for a more convenient one in the other experiments. All materials not already in liquid form were dissolved in water and were allowed to drop from a pipette into the guinea pigs' mouths.

In expressing the sap or juice from white snakeroot, use was made of a small meat chopper. The green weeds were passed through this meat chopper which had been fitted with a plate having small openings. The rotation of the cylinder which fed the machine pressed the macerated weeds through these small openings and at the same time pressed out the plant sap. The sap escaped through the crevice between the rotating cylinder and the frame of the chopper, that is, in the direction opposite that in which the "pomace" or residue escaped.

Post-mortem examinations were made of twenty-two of the thirty-one sheep which died of the disease. Thirty of these animals were observed to tremble in the characteristic manner. With the exception of ewe No. 169 and lamb No. 237, both of which had stomach worms (Hemonchus contortus), there was no evidence that death resulted from causes other than the feeding of E. urticæfolium. Both of these animals manifested characteristic symptoms of trembles, however. Post-mortem examinations were furthermore made of all other animals which died during the course of the experimentation and certain of the internal organs were also preserved for microscopic study. A detailed report covering studies on some of these tissues has been prepared and is representative of all cases examined.

RESULTS OF EXPERIMENTS

a. With Sheep

Experiment 1.—Three ewes, Nos. 11, 26, and 10, were used in Experiment 1, a preliminary experiment which was designed to determine whether harmful effects follow the feeding of E. urticæfolium. This experiment was begun on June 17 and closed on August 2. However, from June 22 to July 6 and from July 18 to July 28, it was impossible to secure the weed. During these periods the animals were grazed on Bermuda grass pasture. Except during the two periods mentioned, a liberal supply of white snakeroot was fed just as it arrived from the point of shipment. In addition, a maintenance ration of grain was fed in a separate trough. Neither the weeds nor the grain were weighed in this experiment. Initial and final weights of each animal were recorded.

In the period between June 17 and July 16, ewe 11 was fed on white snakeroot and grain for an aggregate of fifteen days. A typical case of trembles had developed by July 16, and death occurred two days later. Food was refused during these two days, and there was a decrease in weight from 102 to 77 pounds during the 29 days of intermittent feeding.

Ewe 26 was given a ration of snakeroot and grain for 22 days, between June 17 and August 2. No symptoms of trembles developed during this period. The initial weight of this animal was 91 pounds and the weight at the time the experiment was discontinued was 74 pounds.

The control ewe (No. 10) was maintained on pasture alone from June 17 to July 28. On July 28 she was put in a pen and was given a ration of white snakeroot and grain until her death, which occurred on August 2. This ewe trembled only slightly, was very weak and emaciated, and lost 20 pounds during the experiment. Although the symptoms were not as marked in this case as in ewe 11, yet all conditions indicated that death was due to trembles.

Experiment 2.—Three ewes, Nos. 14, 23, and 26, were employed in Experiment 2. This experiment was planned to confirm the results secured in Experiment 1. Since the animals used in Experiment 1 had refused to eat any considerable quantity of snakeroot when it was fed separately, it was decided to pass the weed through an ensilage cutter and mix it with an equal quantity of weight of grain. One pound of this mixed feed was given each animal twice daily.

On the sixth day after ewe 14 was placed on the experiment she had developed trembles and died on the following day. Her initial weight was 80 pounds and her weight at death was 73 pounds.

The first symptom of trembles in the case of ewe 23 was noted 19 days after the experiment was begun. A well-defined case of trembles developed in this animal, and she died six days after the first symptoms were noticed. Her weight when feeding was begun was 70 pounds, and there was a loss in weight of 8 pounds during the 25 days.

Since ewe 26 had shown no ill effects from the feeding of white snakeroot in Experiment 1, she was used in this experiment. It will be recalled that the weed was fed separately and was not ground in the first experiment. Ewe 26 had eaten only sparingly of the weed in this experiment. However, after 16 days feeding with the mixed ration, a very typical case of trembles developed. The feeding of the weed was therefore discontinued and she was put on pasture.

Experiment 3.—In this experiment, ewes 12, 7, 29, 27, and 19 were fed the mixed ration to determine the amount of weed and the length of time required to develop trembles. Table 1 shows clearly the variation that exists with reference to these two points.

TABLE I—RESULTS OF FEEDING WHITE SNAKEROOT TO SHEEP IN 1916. EXPERIMENT 3.

Ewe No.	*Initial weight.*	*Experiment begun.*	*Feeding discontinued.*	*Days before death occurred.*	*Weight at death.*	*Feed consumed Grain*	*Weed*
	Pounds.				*Pounds*	*Pounds.*	*Pounds.*
12	74	August 5	August 18	13*	....	5.5	11.5
7	86	August 5	September 1	27	61	13	9
29	81	August 5	August 21	16	55	8	10
27	70	August 5	August 10	5	56	2.25	4.5
19	113	August 5	August 23	18	89	8	11.25

*Feeding discontinued after thirteen days.

Ewe 12 was taken off the experiment on August 18, at which time she was affected with trembles. She had lost only 1 pound during these 13 days. It will be noted that the amount of weed required to cause trembles in these five animals varied from 4½ to 11½ pounds and the range in time from 5 to 27 days.

Experiment 4.—In order to determine whether or not trembles is infectious, ewes 26 and 12 were put in a small Bermuda grass lot on August 18. Two healthy ewes from the flock were put in the same lot and all were fed grain in the same trough. It will be recalled that both ewe 26 and ewe 12 had typical cases of trembles when their feeding in experiments 2 and 3, respectively, were discontinued. Ewe 26 died on August 19 and ewe 12 still trembled a week afterward. However, she finally recovered fully. Neither of the other two ewes had developed any symptom of trembles when the experiment was discontinued on September 4, and both subsequently remained normal. It is realized that this indirect evidence of the relationship of a specific infection is in itself not convincing so that in studies to be subsequently reported an effort was made to isolate an organism from the internal organs of affected animals.

Experiment 5.—This experiment was designed to determine the length of time that white snakeroot must be fed to sheep when, after a certain number of days the usual grain ration and pasturage are given. Two animals were therefore fed for three days on a mixture of equal parts of ground weed and grain and were then put on pasture. Two others were fed for six days before being placed on pasture and two others for nine days, after which they were put on pasture. Table II contains the essential facts in this experiment.

TABLE II—RESULTS OF FEEDING WHITE SNAKEROOT TO SHEEP IN 1916. EXPERIMENT 5.

Ewe No.	*Initial weight. Pounds.*	*Final weight. Pounds.*	*Days on experiment.*	*Feed consumed.*	
				Grain.	*Weed.*
169	83	78	3	1.5	1.5
171	85	80	3	1.5	1.5
162	96	82	6	5.5	5.5
168	89	79	6	4.25	4.25
161	102	62	9	6.75	6.75
170	105	92	9	5.5	5.5

Ill effects followed only in the cases of ewes 169 and 161, the former dying 8 days and the latter 11 days after being taken off the experiment. Since ewe 169 evidenced no well marked symptoms of trembles, a post-mortem examination was made which showed that stomach worms may have been a contributory cause of her death. Ewe 161, however, developed a typical case of trembles and is the animal represented in figures A, B, C, and D.

Experiment 6.—The preceding experiments, which were conducted in 1916, had demonstrated the poisonous nature of white snakeroot. Attention was therefore first directed in the succeeding year to determine by experiment, first, if the disease can affect animals in lactation, and second, if these animals can transmit the disease through the milk to their offspring. For this purpose, six ewes with suckling lambs were employed in an experiment which was begun May 13, 1917. The facts brought out in the second part of this experiment are properly referred to Table VI and will, therefore, appear in their appropriate place later in this report. The data bearing on the first point, however, are summarized in Table III, which is presented as follows:

TABLE III—RESULTS OF FEEDING WHITE SNAKEROOT IN 1917 TO EWES WITH SUCKLING LAMBS. EXPERIMENT 6.

Ewe No.	*Initial weight. Lbs.*	*Experiment begun.*	*Date of death.*	*Days before death occurred.*	*Weight at death. Lbs.*	*Gain or loss. Lbs.*	*Feed given.*			
							Grain. Lbs.	*Weed. Lbs.*	*Kale. Lbs.*	*Hay Lbs.*
171	76	May 13	Aug. 30	109	88	12	162	54	134	106
162	81	May 13	Sept. 2*	111	88	7	163	55	134	108
175	83	May 13	Sept. 2	111	98	15	165	55	134	108
167	104	May 13	Aug. 20	99	105	1	132	44	132	96
12	94	May 13	July 24	72	97	3	103	34	134	69
168	80	May 13	July 15	63	74	6	80	27	124	60

*Discontinued feeding white snakeroot at this date.

It will be noted that four of the six ewes developed trembles and died while on this experiment, and that two were apparently normal when the feeding with white snakeroot was discontinued. Only meagre quantities of milk were being given by these animals after July 1. Ewes 168 and 12 died on July 15 and July 24, respectively, at which times their lambs were still alive. Ewe 167, which died August 20, had not suckled her lamb since July 25, and Ewe 171, which died August 30, had not suckled her lamb since June 15.

It should further be noted in this experiment, as well as in experiments 7 and 8 which follow, that the figures representing the quantity of weed given do not represent the quantity actually eaten, since the ewes consistently attempted to avoid eating any of the white snakeroot. Very much smaller amounts would have produced death, had they eaten as much as a pound daily for several days. It is furthermore believed that the liberal quantities of grain, kale, and hay which were given to stimulate the flow of milk, perhaps by absorbing the toxic principle, rendered it less active.

Experiment 7.—It was planned in this experiment to duplicate Experiment 6 in its entirety with ewes in lactation and suckling lambs. The period of lactation was so far advanced, July 7, when the experiment was begun that none of the lambs contracted milksickness, in consequence of which the detailed report of this part of the experiment is withheld. The results of feeding white snakeroot to the mothers, however, will be assembled in Table IV.

TABLE IV—DATA BEARING UPON THE FEEDING OF WHITE SNAKEROOT TO EWES IN LACTATION, IN 1917. EXPERIMENT 7.

Ewe No.	*Initial weight. Lbs.*	*Experiment begun.*	*Date of death.*	*Days before death occurred.*	*Weight at death. Lbs.*	*Gain or loss. Lbs.*	*Feed given.*			
							Grain. Lbs.	*Weed. Lbs.*	*Kale. Lbs.*	*Hay Lbs.*
31	104	July 7	Aug. 28	52	102	—2	77	26	26	52
22	81	July 7	Sept. 2*	56	83	—2	78	27	26	56
17	93	July 7	Aug. 6	30	95	2	39	14	26	30
15	93	July 7	Sept. 2	56	95	2	79	27	26	56
9	94	July 7	Aug. 22*	46	104	10	65	22	26	46
16	135	July 7	Aug. 22*	39	140	5	63	20	26	47

*Feeding was discontinued on the date indicated.

Three of the six ewes employed in this experiment died. Ewe 31 was discontinued on this experiment, however, August 20, and is subsequently reported in Experiment 11, on which she died August 28. Ewe 16 was discontinued on August 15 and is reported in Experiment 9, which resulted in her death August 22. Ewe 17 alone, which died August 6, ate most of the weed given and developed a typical case of trembles, whereas the weed given to the others was for the most part refused. Ewes Nos. 22, 15, and 9 were placed on pasture at the time of discontinuance of the experiment.

Experiment 8.—This experiment was begun on the same date as Experiment 7, in order to determine whether animals not in lactation become affected with trembles earlier than those in lactation. Here again, six animals whose lambs had been weaned were selected. The data bearing on this experiment are presented in the following tabulation:

TABLE V—RESULTS OF FEEDING WHITE SNAKEROOT TO EWES WITH WEANED LAMBS. EXPERIMENT 8.

					Feed given.			
Ewe No.	*Initial weight. Lbs.*	*Experiment begun.*	*Date of death.*	*Days before death occurred.*	*Grain. Lbs.*	*Weed. Lbs.*	*Kale. Lbs.*	*Hay. Lbs.*
25	104	July 7	Sept. 2	56	79	27	24	55
158	108	July 7	Sept. 2*	56	81	27	24	55
149	98	July 7	Sept. 2*	56	81	21	24	55
163	102	July 7	Sept. 2*	56	73	27	24	55
173	105	July 7	Aug. 24	48	68	23	24	47
172	85	July 7	Sept. 7*	56	81	28	24	55

*The feeding of white snakeroot was discontinued at this date.

It may be noted above that ewe 173 died after having been on the experiment 48 days, and ewe 25 after 56 days. Both had typical cases of trembles, whereas the others remained apparently healthy when the feeding with white snakeroot was discontinued, September 2. Since the period of lactation of the ewes in Experiment 7 was so far advanced and since so few deaths occurred in both experiments 7 and 8, no conclusion can be drawn as to the influence of lactation upon the period of resistance.

Experiment 9.—Four lambs and one sheep were used in this experiment to test the toxicity of expressed sap. Lamb 237 was given 500 c.c. in the afternoon of July 30. On the morning of the following day it was given 500 c.c. and an equal quantity in the afternoon. This animal had been given 1500 c.c. and was found dead on the morning of August 1.

Lamb 241 was given 250 c.c. on July 30 and two quantities of 250 c.c. on July 31. She refused to eat oats on the morning of August 1, and was given no more plant juice until the evening of August 3, when she again appeared normal. At this time, she was given 250 c.c. On each of the two succeeding days she was given 500 c.c., making a total of 2,000 c.c. She refused to eat on the morning of August 6 and trembled when made to rise. Feeding with juice was therefore discontinued, and she died on August 9.

Lamb 231 was given daily three feedings of juice of 250 c.c. each beginning on the evening of August 6. Only 250 c.c. were given on August 10 and 500 c.c. on August 11. This lamb had received 4500 c.c. by the evening of August 13, when symptoms of trembles were manifest. Trembling was violent on the following morning and the animal died during the afternoon.

Two feedings of juice of 250 c.c. each were given daily to lamb 240. This test was begun on the evening of August 6 and was discontinued on the evening of August 13. On August 10 only half the quantity was given so that the lamb had consumed 3,000 c.c. by August 13. The animal trembled on the morning of August 14 and the tremors had become so intense by evening that it could not stand. Death occurred during the night of August 14.

Between the evening of August 16 and the evening of August 22, ewe 16 was given a total of 7,250 c.c. of expressed juice. She was sick on the evening of August 22, as noted by the characteristic standing posture. On the following morning, she stood with head lowered and with mouth held open and panted for breath. She seemed to be suffering severe abdominal pain as evidenced by efforts to vomit and constant movement of the hind limbs. Death resulted at about 4 P. M. on August 22.

Experiment 10.—This experiment with lambs 233 and 222 corresponds to Experiment 21 with guinea pigs. Expressed juice was kept on a water bath maintained at a temperature of about 60 degrees C. The decrease in volume due to evaporation was restored by the addition of water. Lamb 333 was given 500 c.c. daily in two feedings beginning on the evening of August 6. None was given on August 11. She was first noticed to tremble on August 17 and feeding was discontinued. This animal was given a total of 5,000 c.c. Death took place August 18. The feeding of lamb 222 was begun at the same time, she being given 750 c.c. daily except on August 11, when none was given. Trembling was first noted on the morning of August 25 and she died during the night of August 29. No juice was given after August 24, and she received a total of 9,250 c.c.

Experiment 11.—The residue from which the volatile oil had been distilled was given to ewe 31 beginning August 20. By the evening of August 22, 3,800 c.c. had been given. None was given on the two succeeding days, and at noon on the 25th trembling was noted. Death occurred during the night of August 27.

b. With Hogs

Experiment 12.—The purpose of this experiment was to determine whether hogs are subject to ill effects following the feeding of white snakeroot. Accordingly, two animals were given this weed along with a ration of grain and shorts beginning June 21. They consistently refused to eat any considerable quantity of the weed even though it was mixed with moistened grain and shorts. One animal, however, contracted trembles on September 24 and died six days later. This animal is shown in figures I, J, K, and L in the accompanying illustrations. The other animal remained unaffected.

c. With Guinea Pigs

Experiment 13.—On June 26, three guinea pigs were placed on an experiment which was designed to show whether guinea pigs are subject to poisoning by the ingestion of green leaves of white snakeroot. No. 1 aborted on July 1, refused all feed on that day and on the following day, and was found dead on the morning of July 3. The autopsy showed that death very probably resulted from abortion. No. 2 also aborted on July 1, but her appetite remained quite normal until her death which occurred on July 4. She was very inactive, however, during this period, but exhibited no other indication of being affected. Abortion resulted on June 29 in the case of No. 3. She ate sparingly during suceeding days and remained humped up with eyes partially closed. Upon being disturbed, she appeared quite active. On July 6 a period of coma began and death resulted on July 9. Two more animals, No. 4 and No. 5, were placed in this cage on July 3. On July 9 No. 4 was very sick and breathed with difficulty. She became comatose and was found dead on the morning of July 11. No. 5 appeared normal until July 10 and was very inactive thereafter until her death on July 23.

Experiment 14.—Beginning June 26, two male guinea pigs were fed on green stems of white snakeroot to determine if stems are as poisonous as leaves. No. 1 was noted to be droopy on the morning of July 3 and was dead forty-eight hours later. No. 2 was first noted to be affected on July 7. He then appeared listless, refused entirely the green stems and ate only sparingly of the grain. By July 9 respirations were labored, and he sat crouched in the corner of his cage. His eyes were half-closed, his ears drooped, and his head drooped. By July 11 his body was limp and he had so lost control of his legs that when lain on his side, he could not regain his feet. At this stage, trembling was apparent. The symptoms exhibited in No. 2, which animal is shown in Fig. H, are typical of trembles in guinea pigs as developed throughout these experiments. This animal was asphyxiated on July 11 and an autopsy immediately made.

Experiment 15.—At the same time that the above experiments were begun to determine whether the leaves and stems are toxic to guinea pigs, the feeding of two animals on an aqueous extract was also begun. On June 28, some diarrheal symptoms were present, which persisted until July 5. They ate ravenously, however, and by July 8 had eaten the water soluble material from 1800 grams of dry white snakeroot with no ill effect except the disgestive disturbance mentioned. For the next fourteen days they were given an aqueous extract prepared from the fresh weed. At the end of this time, they were in good condition and the feeding was discontinued. It does not appear from this that the active principle is water soluble, or if it is, the heat of desiccation destroys its toxicity. It will be recalled in this connection, that the feeding of aqueous extracts previously reported by Crawford (1908) were not followed by trembles.

Experiment 16.—The feeding of three guinea pigs with material extracted with dilute hydrochloric acid was begun on June 28. This extract was made neutral with sodium hydroxid with the consequent precipitation of salt. No. 1 was sick on July 2, was very inactive on July 3, but ate of the grain and clover. He refused to eat on the morning of the following day, and died at noon. The other two were still unaffected on July 8 when feeding was discontinued.

Experiment 17.—The feeding of alcohol soluble products was begun on the evening of July 3. By the evening of July 5, two guinea pigs had eaten the extract from 1,800 grams of dry weed. No ill effects whatsoever followed during the succeeding two weeks in which they were kept under observation.

Experiment 18.—In this experiment six guinea pigs were given by means of a pipette the expressed sap or juice of white snakeroot. On the evening of July 20, two large animals, each weighing nearly 800 grams, were given 10 to 12 c.c. apiece. On the following morning both were noted to be droopy and refused to eat. No. 1 was so weak that he could scarcely walk and seemed unable to control the movements in the hind limbs. Each was given about 12 c.c. on the morning of the 21st and an equal quantity at noon. At 5 P. M. No. 1 died, having been given a total of about 35 c.c. No. 2 was given two feedings on the 22d and had received a total of about 50 c.c. during the three days of feeding. He became more inactive and listless daily, passed into a state of coma and died July 26.

On the morning of the 24th two more guinea pigs were placed in this cage and each was given in three feedings about 25 c.c. Both were sick on the following morning, but were given during the day three quantities of expressed juice totaling about 25 c.c. for each animal. On the following morning No. 3 died. No 4 was fed three times daily on the 26th, 27th, and 28th. Feeding was discontinued on this last date and he died three days later.

On the afternoon of the 26th another animal, No. 6, was placed in this cage, was given 16 to 20 c.c. daily in two feeds and died on August 14. No. 6 remained quite normal throughout this entire period except for two or three days prior to death.

Experiment 19.—In this experiment the expressed sap was reduced to dryness over a water bath, the water in which was kept boiling. On July 11 one animal was placed on this experiment, and given daily the equivalent of 25 to 30 c.c. of freshly expressed juice. No ill effects had appeared on July 20, when feeding was discontinued.

This animal along with two others was again placed on the experiment on July 26. Each was given daily quantities equivalent to 25 or 30 c.c. of fresh sap. Since no ill effects had appeared by August 14, the feeding was discontinued. When it is remembered that the sap was toxic before it was desiccated and that each animal had received the equivalent of many times more of fresh juice than was necessary to produce death, it is evident that desiccation at high temperature either destroys the toxicity or that the active principle had been volatilized.

Experiment 20.—This experiment was designed to show whether a toxic volatile principle is present in the sap of white snakeroot. Accordingly, 5,800 c.c. of expressed sap were distilled in a flask over an open flame. A turbid distillate was collected which contained a very pungent volatile oil. This distillate was fed to three guinea pigs beginning August 14 and concluding August 29. No ill effects whatsoever were apparent. The materials which remained after distillation were fed to ewe 31 and have been reported as Experiment 11 along with the feeding experiments with these animals.

Experiment 21.—The expressed sap fed to the three animals on this experiment had been kept for forty-eight hours on a water bath maintained at 55 to 60 degrees C. This temperature was found to be sufficient to drive off the volatile oil and was believed to be hot enough to prevent fermentation yet not a sufficient degree of heat to change the chemical constitution of the plant juice. The feeding of two animals, thrice daily, was commenced at noon on July 30 and of another on August 3. One of the first two was observed to be sick on August 2. This animal died on the afternoon of August 4. The other two continued to eat their food, however, and were merely less active than normal individuals throughout the remainder of the time that they were maintained on this product or until August 23.

TRANSMISSION OF THE DISEASE.

a. By Milk and Milk Products

In sections where milksickness prevails, it is firmly believed the disease is acquired through the use of milk from cattle affected with trembles. There seems, moreover, to have been little doubt of this fact in the minds of early settlers in the Middle West and more or less evidence has been presented in published accounts by McCall (1822), Lewis (1829), Barbee (1840), Logan (1849), Yandell (1852), Philips (1857), Cosby (1866), Nichols (1876), Woodfin (1878), Sykes (1891), Beck (1905), and McCoy (1907), in the several medical journals.

An instance of an apparent exception came under the observation of Jordan and Harris (1909) during the summer of 1908. Four cases of milksickness in a family of ten developed near Altamonte, Ill., on a farm which had "long been known to give rise to milksickness." In discussing the occurrence, they state that "The cows were to all appearance entirely healthy, and showed no signs of disease before or after the outbreak; a six-to-eight weeks old calf whose mother was one of the animals providing the milk used in the family also seemed in the best of condition. A cat that was being fed on the milk from these cows was itself well, but lost all of its eight-weeks-old kittens, five in number, during the outbreak in the family. A dog receiving milk from the same cattle showed signs of illness and was noticed to vomit repeatedly."

This case does not entirely agree with the claim quite commonly advanced in "milksick" sections in the State to the effect that so long as cows are being milked or their calves are suckling, they show no signs of trembles. The calves, however, may sicken and die or persons who use the milk may be affected with milksickness. In due time, however, the milk is suspected and in consequence, the cows are left to go dry and then develop the disease. This peculiarity furthermore finds support in the writings of Drake (1841), Graff (1841), and Kimmel (1891). It is further strengthened by the common observation among farmers that in the herd of cows, heifers, and steers, the heifers and steers become affected earlier than cows in lactation.

To Lambs.—Experiment 22.—The present experiments bearing upon the acquisition of milksickness through the milk deal with suckling lambs whose mothers were fed white snakeroot as previously recorded in Table III. The mothers of the lambs, the data on which lambs are recorded in Table VI, were also well advanced in the period of lactation as were those reported in Table IV. This fact is believed to be the potent factor in determining the small number of cases of milksickness which developed.

TABLE VI—RESULTS OF TRANSMISSION OF THE DISEASE THROUGH THE MILK FROM EWES TO LAMBS. EXPERIMENT 22.

Animal No.								*Feed given.*	
Ewe No.	*Lamb No.*	*Initial weight. Lbs.*	*Experiment begun*	*Date of death.*	*No. days on experiment.*	*Weight at death. Lbs.*	*Gain or loss. Lbs.*	*Grain. Lbs.*	*Kale. Lbs.*
171	244	38	May 13	June 17	35	27.5	—10.5	9	33
162	243	50	May 13	July 25*	73	61	11	30	71
175	242	40	May 13	June 17	35	39	—1	15	32
167	241	33	May 13	July 25*	73	32	—15	36	71
12	237	46	May 13	July 25*	73	56	10	35	71
168	200	48	May 13	July 15*	83	45	—3	35	60

*These lambs were put on pasture on the dates indicated.

Two lambs, Nos. 244 and 242, developed genuine cases of milksickness and died thirty-five days after the experiment was begun, June 17. Ewe 171, the mother of lamb 244, showed no evidence of trembles until a few days before her death, which occurred, as noted before, on August 30. These individuals are shown in Fig. G, at a time when the lamb had passed into a state of coma. The mother of lamb 244 never manifested any evidence of trembles although she was continued on the experiment until September 2. This fact accords with the observation which has previously been made by Jordan and Harris (1909) that animals can transmit the disease through the milk without themselves ever manifesting any signs of the disorder.

Lambs 237 and 241 were employed after this experiment was concluded in tests on the toxicity of expressed sap as has been reported in Experiment 21. The other two lambs, Nos. 243 and 200, were placed on pasture.

The butter from cows affected with trembles is also generally believed to be the source of milksickness. A number of publications dealing with this phase of the subject have appeared in which the authors, among whom are Johnson (1866), Gray (1881), Beach (1883), Scott (1889), Beck (1905), and Jordan and Harris (1909), commit themselves to this belief. The last named writers describe an outbreak which came under their observation at Morris, Ill., in 1908. Five cases developed in a family which secured butter from a farm six miles distant. The cows which gave the milk from which this butter was made, ranged in woodland in which eight cattle had contracted trembles two years earlier. About a month after the recovery of these five persons, a typical case appeared in one of the young men on the farm from which the butter had been procured. In concluding this account, they state that "There seems little doubt, therefore, in view of all the facts, that butter brought from the 'milksick' farm was responsible for the cases."

To Mice.—Experiment 23.—Opportunity has not been afforded in the present experimental study to secure conclusive data upon butter as an agency in the transmission of milksickness. Three mice were fed butter made from the milk of a cow feeding upon white snakeroot. One died about two weeks after feeding was begun, another a week later, while the other one remained unaffected. Aside from general debility, no other symptom was prominent in these two animals, and the cause of their death could not be determined with certainty. It is reasonable to conclude, however, in the light of experiments on milk as a carrier of milksickness, that the disease may be transmitted through butter.

No clear-cut evidence pointing to a relationship between cheese and the development of the disease appears in available medical literature. Some few writers (Scott, 1889) assume that it is a generally accepted fact and in view of the evidence that milk and butter are capable of transmitting the disease, it is logical to suppose that cheese may also be a carrier. No feeding tests, however, have ever been directed toward the settlement of this claim.

b. Through the Agency of Meat

Numerous records exist of the transmission of this disease through the eating of flesh of animals affected with trembles. Coleman (1822), Lewis (1829), Sale (1891), and Conner (1904) cite specific instances of the acquisition, by man, of this disease from meat. Yandell (1867) has included in his account statements which are contrary to this idea. Statements apparently from eye-witnesses (Coleman, 1822; De Bruler, 1858) have, moreover, been made to the effect that carnivorous animals may acquire the disease from the flesh of animals dead of trembles. More remarkable than this, however, is the statement that these animals can transmit the disease to others indefinitely. Instances like the following from McCall's (1830) report illustrate the point in question. Some sheep which died of trembles were eaten by hogs, which in turn, succumbed to the same disease. The carcasses of all of the hogs except one were burned. Chickens which ate of this one were eaten in turn by persons who subsequently developed cases of milksickness. Another similar record by De Bruler (1858) states that a cow gave the disease to her calf, whose body was eaten by dogs. The dogs in turn died of trembles and a pet crow which fed on the dogs' flesh also died.

The more recent experiments by Jordan and Harris (1909) in which four dogs were fed upon flesh of animals dead of trembles resulted in the production of no ill effects.

To Dogs.—Experiment 24.—These results with meat are in accord with the results secured in the present studies. During 1916, the carcasses, including livers, hearts, kidneys, and spleens of three sheep dead of trembles were fed to three dogs with no resultant ill effects. During the next season, four dogs were given the bodies of eight animals with no manifestation of trembles. It can only be suggested with reference to the observations of others to the effect that carnivorous animals may die after having eaten the flesh of an animal dead of trembles, that ptomaines may have been present as the result of bacterial growth. This was guarded against in the present experiments by placing the carcasses, immediately after the autopsies had been performed, in a refrigerator. Daily portions were then given sufficient to satisfy the dogs' hunger.

SUMMARY OF FEEDING EXPERIMENTS.

During the course of the feeding experiments with white snakeroot, a total of 41 sheep and lambs have been subjected to experimental conditions. Three others died in the experimental work with sodium compounds, to be reported later in this paper. Twenty-eight of these died and all except two were observed to have been characteristically affected with trembles or milksickness. These two were infested with stomach worms, which infestation may have been a contributory cause of their death. Two lambs contracted milksickness from the milk of ewes feeding upon white snakeroot.

Two hogs were fed white snakeroot. One of these animals contracted a fatal case of trembles and the other remained apparently unaffected.

Twenty-nine guinea pigs, fifteen of which succumbed on the experiments, were given white snakeroot or some of the products prepared from this plant.

Seven dogs ate of the flesh of sheep dead of trembles with manifestly no ill effects.

Three mice were given butter from the milk of a cow feeding upon white snakeroot. Two of them died while the other remained unaffected.

POST-MORTEM EXAMINATIONS

No detailed studies of the pathological anatomy of animals dead of trembles or milksickness, other than those of Jordan and Harris (1909) appear in the voluminous literature on this disease. These authors give a general historical account of autopsies by other writers. These accounts give the impression of being fragmentary and indicate a lack of familiarity with anatomy in general and of experience with making post-mortem examinations. Such statements as the following indicate the point in question.

Bennett (1822) says that the stomach contents of cattle is dry and that the liver has a morbid appearance. Beck (1822) describes the heart and aorta as greatly enlarged and their inner coat sloughy. Miller (1867) asserts that gastric and hepatic lesions accompany the disease. Hibberd (1844) mentions the finding of a diminution of the caliber of the stomach and intestine in all of the cases examined. Mendenhall (1861) describes the inner coat of the stomach and intestine as being gangrenous. Newman (1867) records the singular observation that "in examining a milk cow after death the skin was found very much thickened and covered with small pustules." Dicky (1852) states that the peritoneum and small intestine showed signs of inflammation. Graff (1841) says that there is evidence of cerebral inflammation, of a similar condition of the stomach and small intestines, that the spleen is enlarged and the liver softened. Way (1893) noted congestion of the cerebral meninges in the case of a six-weeks-old calf.

Jordan and Harris (1909) had opportunity to perform autopsies upon several cattle, two lambs, and one horse. The following account of the microscopical appearance of these animals covers their findings. Externally the body presents nothing on inspection that is worthy of note. There may be present in the pleural cavities a small quantity of clear, yellow fluid. On opening the thoracic cavity, the lungs do not collapse. A quantity of frothy fluid exudes from the sectioned surface of the lungs. As a rule, the pericardial sac contains varying quantities of clear, straw-colored fluid which coagulates when exposed to the air. Numerous ecchymotic areas which are more numerous at the base of the heart, occur along the course of the cardiac veins. There was noted on section of the heart either fatty areas or streaks, or cloudy swelling.

The peritoneal cavity contains no abnormal amount of serous fluid. The liver is uniformly enlarged and of a purple color, being full of blood. The consistency of the liver substance is soft and friable, and of the "nutmeg" appearance. Blood drips freely from the sectioned surface. The parenchymatous tissue is fatty. The kidneys are always enlarged and engorged with blood. Cloudy swelling of the parenchyma and in some cases slight fatty changes are noted. The bladder may be injected. In some cases, the urine is cloudy and apparently contains blood. The mucosa of the small intestines may show injection and ecchymoses. The meningeal vessels of the brain appear congested.

In the microscopical examination, these authors find that the most profound pathological changes occur in the heart, liver, and kidneys. In the heart there may be noted cloudy swelling and fatty changes. In the liver the parenchymatous cells appear swollen with more or less cloudy swelling and fatty metamorphoses. The central veins are distended with blood. The kidneys show hyperemia, glomerulitis, and parenchymatous degeneration.

The following is a detailed report of seven representative cases in sheep and lambs.

CLINICAL DATA ON CASE 1, TABLE VII, SHOWING TEMPERATURE, PULSE, AND RESPIRATION OF EWE 168.

	Temperature.			*Pulse.*			*Respiration.*			*Atmospheric Temperature.*			*Character of Day*
Date.	A. M.	M.	P. M.	A. M.	M.	P. M.	A. M.	M.	P. M.	*Max.*	*Min.*	*Mean*	
5–11	......	......	103.6	......	......	63	......	......	30	70	47	58	Cloudy.
5–12	......	......	103.8	......	......	60	......	......	42	61	46	54	Partly cloudy.
5–13	......	102.1	......	......	60	......	......	48	......	60	42	51	Partly cloudy.
5–14	102.1	101.2	102.0	66	69	54	48	43	30	66	43	54	Partly cloudy.
5–15	101.4	101.3	103.5	60	54	66	42	36	48	76	49	62	Clear.
5–16	102.0	102.0	101.9	60	66	66	45	42	48	80	57	68	Cloudy.
5–17	101.7	101.9	102.1	60	63	66	30	45	48	85	62	74	Clear.
5–18	101.4	102.0	102.2	63	60	60	36	39	45	87	65	76	Clear.
5–19	101.8	101.6	102.6	69	66	66	42	48	54	92	64	78	Clear.
5–20	101.6	102.1	102.6	54	60	66	36	54	57	90	65	78	Clear.
5–21	101.6	102.0	102.7	72	75	72	33	42	60	90	64	77	Clear.
5–22	102.1	102.2	102.7	66	54	60	42	42	66	88	62	75	Clear.
5–23	101.5	102.8	102.8	54	66	60	30	42	42	76	55	66	Partly cloudy.
5–24	100.6	101.6	102.6	60	63	60	27	33	33	72	49	60	Clear.
5–25	101.4	101.0	102.2	66	69	69	27	33	36	77	55	66	Partly cloudy.
5–26	101.2	102.0	102.6	57	60	72	30	30	36	77	53	65	Partly cloudy.
5–27	101.7	102.0	102.6	66	72	63	27	42	36	86	64	75	Cloudy.
5–28	101.7	101.3	102.6	66	57	60	30	30	42	80	68	74	Cloudy.
5–29	101.4	......	101.8	66	......	60	48	......	36	78	59	68	Clear.
5–30	101.3	101.6	101.5	60	60	66	30	36	42	82	55	68	Clear.
5–31	101.8	102.4	101.5	66	66	60	36	36	30	78	59	68	Cloudy.
6– 1	101.3	103.0	102.8	66	66	63	36	42	54	89	66	78	Partly cloudy.
6– 2	102.0	102.4	103.3	75	66	69	42	48	54	90	65	78	Clear.
6– 3	102.2	102.6	103.4	69	57	66	48	72	60	91	66	78	Partly cloudy.
6– 4	101.8	102.4	102.6	66	69	72	36	36	42	73	64	68	Cloudy.
6– 5	101.9	101.9	102.4	66	69	78	36	33	42	85	64	74	Partly cloudy.
6– 6	102.2	102.0	103.0	72	72	66	36	42	54	89	67	78	Partly cloudy.
6– 7	101.8	102.3	103.0	60	66	63	30	45	54	84	70	77	Partly cloudy.
6– 8	102.0	102.8	102.6	66	66	63	36	39	36	86	64	75	Partly cloudy.
6– 9	101.6	102.0	102.2	72	63	59	42	66	54	86	64	75	Partly cloudy.
6–10	101.6	101.4	101.5	66	66	63	30	30	27	76	64	70	Cloudy.
6–11	101.2	101.5	102.2	63	66	66	33	33	30	83	65	74	Partly cloudy.
6–12	101.4	102.2	102.8	75	60	57	36	30	54	88	67	78	Clear.
6–13	101.6	101.4	102.2	78	57	60	39	30	48	88	68	78	Partly cloudy.
6–14	101.5	101.8	102.0	63	60	66	27	36	27	84	68	74	Cloudy.
6–15	100.7	102.2	102.4	68	60	57	33	30	27	78	62	70	Partly cloudy.
6–16	101.6	101.2	101.6	60	57	57	30	36	27	73	56	64	Clear.
6–17	99.6	100.3	101.8	60	63	63	42	36	36	76	54	65	Clear.
6–18	100.5	101.2	102.0	66	72	57	42	30	39	82	58	70	Clear.
6–19	100.8	101.2	101.8	51	54	60	36	30	45	80	60	70	Cloudy.
6–20	101.8	102.0	102.4	60	60	63	36	39	66	85	63	74	Clear.
6–21	101.4	102.2	102.4	60	66	60	48	42	66	88	64	76	Partly cloudy.
6–22	100.8	101.0	101.6	66	66	63	30	48	54	85	63	74	Clear.
6–23	101.8	102.2	102.4	54	60	63	24	54	60	91	69	80	Clear.
6–24	102.0	103.0	103.5	66	72	60	48	84	72	93	67	80	Clear.
6–25	102.1	102.6	103.3	66	75	60	48	81	84	88	68	78	Clear.
6–26	101.6	102.0	103.5	72	72	66	57	72	90	91	72	82	Partly cloudy.
6–27	102.6	102.2	103.4	72	69	66	60	84	96	92	71	82	Clear.
6–28	102.0	102.4	102.6	75	66	69	54	72	84	90	69	80	Partly cloudy.
6–29	101.6	101.8	102.4	72	66	69	48	60	48	86	67	76	Cloudy.
6–30	102.2	101.8	102.6	66	63	63	36	54	54	84	65	74	Partly cloudy.
7– 1	101.8	102.2	102.5	60	60	57	48	48	63	87	67	77	Clear.
7– 2	102.0	102.2	102.9	60	66	63	33	54	54	88	67	78	Partly cloudy.
7– 3	102.2	101.8	102.3	60	63	60	36	45	66	85	67	76	Cloudy.
7– 4	102.2	102.0	102.1	60	60	63	39	54	36	79	65	72	Cloudy.
7– 5	101.9	102.2	103.0	60	66	69	51	66	66	85	63	74	Partly cloudy.
7– 6	102.0	102.3	102.0	60	66	63	48	57	48	83	69	76	Partly cloudy.
7– 7	102.0	102.6	102.8	63	66	66	42	42	36	81	69	75	Cloudy.
7– 8	102.0	101.8	101.8	54	66	60	36	60	48	86	68	78	Partly cloudy.
7– 9	102.3	101.9	102.6	72	63	66	42	48	72	89	70	70	Clear.
7–10	102.2	102.6	103.0	66	63	60	48	48	54	88	67	78	Partly cloudy.
7–11	102.2	103.2	102.7	51	54	60	36	48	42	83	66	74	Clear.
7–12	102.4	102.5	102.4	57	60	66	42	54	54	92	67	80	Partly cloudy.
7–13	101.6	101.6	102.2	63	66	72	30	36	57	86	67	76	Partly cloudy.
7–14	101.4	101.5	102.4	75	72	78	42	48	48	86	67	76	Partly cloudy.
7–15	104.2	Died	......	69	......	......	42	......	......	90	69	80	Clear.
7–16	......	......	......	......	......	......	......	......	......	91	69	80	Clear.
7–17	......	......	......	......	......	......	......	......	......	90	67	78	Partly cloudy.
7–18	......	......	......	......	......	......	......	......	......	82	66	74	Cloudy.
7–19	......	......	......	......	......	......	......	......	......	78	66	72	Cloudy.
7–20	......	......	......	......	......	......	......	......	......	79	68	74	Cloudy.
7–21	......	......	......	......	......	......	......	......	......	80	69	74	Cloudy.
7–22	......	......	......	......	......	......	......	......	......	86	71	78	Partly cloudy.
7–23	......	......	......	......	......	......	......	......	......	89	72	80	Partly cloudy.

These data show that in ewe 168 there were no marked abnormalities in temperature, pulse, and respiration. There has been included with this table a statement of the meteoric conditions since they exert an influence upon the temperature, pulse, and respiration of animals.

Clinical Notes. The first symptoms appeared July 13, when the respiration was slightly jerky. On the 14th, she lay with head and neck extended and jaw resting upon the ground. After standing for about a half-minute, she would begin to tremble as noted in all the other cases. As soon as trembling began, she would lie down. In the afternoon the symptoms were more aggravated. She went into a state of coma during the night before she died, and death occurred July 15, at about 8 A. M.

Blood Study. On May 18 a study was made of the blood of ewe 168 to determine the normal condition prior to the feeding of white snakeroot. The following is the result of this study:

Numerical count:	
Erythrocytes	8,040,000
Leucocytes	14,000
Differential count:	
Neutrophiles	46 per cent
Lymphocytes, large	4 per cent
Lymphocytes, small	45 per cent
Eosinophiles	2 per cent
Mast cells	3 per cent
Hemaglobin	72 per cent

On July 14 a study of the blood was made with the following result:

Numerical court:	
Erythrocytes	9,540,000
Leucocytes	9,000
Differential count:	
Neutrophiles	45 per cent
Lymphocytes, large	14 per cent
Lymphocytes, small	39 per cent
Mast cells	2 per cent
Hemaglobin	72 per cent

There was thus a slight increase in the small lymphocytes at the expense of the large lymphocytes, a slight increase in the erythrocytes, and a numerical decrease in the leucocytes. The hemaglobin remained normal.

Protocol. Externally the body presents nothing worthy of note.

The Thoracic Cavity.—The lungs appear normal and collapse when the thorax is opened. There is neither pneumonia, broncho-pneumonia, nor pleuritis. The color of the lung is pale salmon. No excess of fluid is found in the thoracic cavity.

The Heart.—There is an excessive amount of pericardial fluid. Petechiation of the epicardium in the region of the coronary arteries and at the base of the heart is noted. The heart is filled with dark, coagulated blood. There are no agony thrombi and the muscles appear pale in color.

The Abdominal Cavity.—An excessive amount of serous fluid exists in the abdominal cavity. Muco-enteritis involving the abomasum and duodenum and a portion of the jejunum is present. Stomach worms are found in the abomasum. The small and large intestines are affected with nodular disease. The large intestines otherwise appear normal.

The Liver.—The liver weighs one pound thirteen and one-half ounces. It is of a dark metallic color and is congested so that blood drips from the sectioned surface. The gall bladder is filled with a rather dark, tarry bile. Cloudy swelling is evidently present.

The Kidneys.—Both kidneys weigh four and one-half ounces. Externally they present a metallic color. Both active and passive congestion and cloudy swelling are present. The glomerules can be noted as minute, red pin point-like spots in the renal cortex.

The Bladder.—Empty and apparently normal.

The Musculature.—Normal.

The Brain.—Congested.

The Lympth Glands.—Normal.

Microscopical Examination. *The Liver.*—Both active and passive congestion, cloudy swelling and fatty degeneration are noted.

The Kidneys.—Both active and passive congestion are present. Glomerulitis is noted and some of the convoluted tubules show evidence of fatty degeneration.

The Brain.—The blood vessels are congested. An occasional polymorphonuclear leucocyte and plasma cells occur throughout the tissues. The brain cells are not shrunken and appear normal.

The heart.—The heart muscle fibers have lost their cross striation and many areas show fatty degeneration. The nuclei stain deeply, in fact, may be considered picnotic. The blood vessels are congested:

The Lungs.—Normal.

The Lymph Glands.—Normal.

The Pancreas.—Normal.

The Adrenal Glands.—Normal.

The Duodenum.—The duodenum is infiltrated with round cells.

The Blood Vessels.—Congested.

CASE 2, TABLE VIII, SHOWING TEMPERATURE, PULSE, AND RESPIRATION OF LAMB 200.

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
5–11	....	....	103.1	80	....	80	......	......	112
5–12	....	....	104.0	....	....	72	......	......	96
5–13	....	103.2	....	....	72	....	79	......	......
5–14	103.2	....	102.8	78	86	72	72	87	54
5–15	102.9	102.9	103.1	78	90	84	66	108	90
5–16	103.3	102.9	103.3	84	84	79	90	120	114
5–17	103.7	103.6	103.4	78	84	78	126	120	132
5–18	103.6	103.2	103.0	84	96	88	132	126	132
5–19	104.0	104.0	103.4	78	78	78	126	142	132
5–20	103.6	103.7	103.9	84	84	78	139	156	156
5–21	103.8	103.0	103.9	78	84	87	138	144	158
5–22	104.0	103.4	103.4	84	78	84	114	114	132
5–23	103.6	103.8	103.7	84	84	84	138	144	147
5–24	103.2	103.2	103.2	84	84	78	78	90	102
5–25	103.0	103.0	103.4	72	78	84	90	96	108
5–26	103.2	103.0	103.1	87	84	84	90	78	138
5–27	103.8	103.6	103.4	84	72	72	108	114	102
5–28	103.2	103.6	103.8	72	66	72	72	90	108
5–29	103.0	....	103.9	81	....	78	72	......	90
5–30	102.7	103.0	103.7	72	84	72	60	108	78
5–31	103.0	103.1	103.7	69	72	78	66	84	90
6– 1	104.0	103.9	103.9	84	69	72	120	126	108
6– 2	103.0	103.3	103.4	78	69	72	108	126	138
6– 3	104.0	104.0	103.9	72	81	72	120	114	126
6– 4	103.0	103.7	103.8	75	84	84	84	96	120
6– 5	103.2	103.6	103.7	78	84	81	105	102	102
6– 6	103.2	103.6	103.9	75	72	87	114	102	108

	Temperature.			Pulse.			Respiration.		
Date.	A.M.	M.	P.M.	A.M.	M.	P.M.	A.M.	M.	P.M.
6– 7	103.4	103.7	104.2	72	75	81	108	108	102
6– 8	103.9	104.0	103.8	84	75	90	96	108	126
6– 9	102.8	103.0	103.2	72	84	75	84	90	78
6–10	103.3	103.8	103.7	72	84	84	54	87	90
6–11	103.4	103.8	103.8	78	87	81	96	114	114
6–12	103.4	103.8	103.8	81	78	82	114	120	132
6–13	103.8	104.2	104.2	72	72	75	96	96	120
6–14	104.3	104.1	103.8	81	75	60	114	120	102
6–15	103.1	104.0	104.7	72	66	66	66	90	96
6–16	102.8	103.3	104.0	78	72	60	90	60	60
6–17	102.4	103.5	104.2	60	60	54	72	66	60
6–18	103.3	103.0	103.0	63	60	60	84	90	102
6–19	103.2	103.5	103.8	60	57	66	54	72	78
6–20	103.1	104.1	104.2	72	60	72	90	90	96
6–21	103.4	104.2	104.2	69	75	63	102	90	96
6–22	103.4	103.4	104.1	72	69	78	78	108	78
6–23	103.9	104.5	104.0	69	72	75	60	90	108
6–24	104.0	104.1	104.0	72	78	72	90	120	114
6–25	103.7	103.4	104.0	60	72	69	90	90	108
6–26	103.5	104.0	104.2	69	66	66	96	90	120
6–27	103.6	104.0	104.4	69	72	60	120	120	102
6–28	102.9	104.0	103.9	63	61	68	60	108	114
6–29	104.2	103.6	104.0	66	72	60	90	96	78
6–30	103.1	103.5	103.5	66	72	72	54	90	96
7– 1	103.6	103.6	104.0	54	66	57	66	90	78
7– 2	103.6	104.2	104.0	60	72	60	78	96	96
7– 3	103.8	103.4	103.4	60	69	54	54	75	36
7– 4	103.2	103.4	103.9	66	60	60	66	48	72
7– 5	103.4	103.4	103.8	54	66	69	48	78	108
7– 6	103.2	103.6	103.5	66	66	72	66	84	90
7– 7	103.4	103.8	103.7	72	72	66	90	84	90
7– 8	103.2	103.9	103.8	54	57	60	48	84	108
7– 9	103.2	103.6	103.6	57	51	57	66	66	90
7–10	103.7	104.0	104.1	63	60	66	84	108	144
7–11	103.1	103.0	104.0	60	54	54	36	48	72
7–12	103.5	103.9	104.3	60	60	66	78	78	90
7–13	103.3	103.8	104.1	66	66	69	73	72	90
7–14	103.2	103.7	104.6	54	60	69	66	84	84
7–15	103.5	103.5	103.8	60	63	60	66	84	96
7–16	103.4	104.1	104.0	60	66	60	78	90	96
7–17	103.8	103.9	103.9	66	60	72	108	72	84
7–18	103.8	104.0	103.0	66	66	69	54	84	78
7–19	103.4	104.0	103.4	69	63	66	72	84	78
7–20	104.7	103.8	103.6	72	69	60	90	72	72
7–21	103.8	104.4	103.8	66	63	69	102	90	84
7–22	103.9	103.9	103.5	66	69	66	96	102	108
7–23	104.2	104.3	103.7	57	60	66	102	78	168
7–24	103.9	103.8	103.6	60	66	66	84	114	108
7–25	103.7	104.0	103.8	69	69	72	90	96	90
7–26	103.8	103.9	103.6	66	66	72	108	114	102
7–27	103.8	104.2	104.0	60	72	72	102	114	114
7–28	104.4	103.8	104.4	72	75	78	102	102	120
7–29	103.9	104.2	104.3	69	72	72	84	114	114
7–30	105.0	104.7	104.8	69	72	66	108	114	132
7–31	104.9	105.0	105.1	72	66	72	108	114	120
8– 1	104.7	104.8	105.0	69	72	69	120	126	120
8– 2	104.7	104.8	105.1	72	69	72	120	114	120
8– 3	103.8	103.9	104.4	78	84	81	66	108	108
									Died

These data show a slight disturbance in temperature and pulse and a very considerable increase in respiration.

Clinical Notes. The feed consisted of one-half pound of oats and one-fourth pound of weeds given twice daily and one pound of kale at noon. Feeding was begun July 16, and during this experiment this animal received 17 pounds of oats, 8.7 pounds of white snakeroot, and 17 pounds of kale. The first symptoms were noted August 1, when she appeared to be sluggish, with loss of appetite. On August 3 she ate sparingly. After standing about three-quarters of a minute she began to tremble and would soon lie down. Respirations were accompanied by groaning. At noon, the trembling was more violent than in the morning and she could not remain standing as long. During the afternoon she lay with neck outstretched and jaw resting upon the ground. She was found dead the next morning.

Blood Study. On May 18 a study was made of the blood to determine its normal condition. The following are the results:

Numerical count:	
Erythrocytes	13,760,000
Leucocytes	11,000
Differential count:	
Neutrophiles	19 per cent
Lymphocytes, large	5 per cent
Lymphocytes, small	72 per cent
Eosinophiles	4 per cent
Hemaglobin	77 per cent

On August 3, after severe symptoms had developed, another study was made. The results are as follows:

Numerical count:	
Erythrocytes	15,520,000
Leucocytes	7,000
Differential count:	
Neutrophiles	59 per cent
Lymphocytes, large	13 per cent
Lymphocytes, small	28 per cent
Hemaglobin	78 per cent

There was an apparent increase in the erythrocytes and an increase in neutrophiles at the expense of lymphocytes. There was a marked decrease in the total number of leucocytes. The decrease may have been in the lymphocytes, in which case there would be an apparent increase in the neutrophiles over the lymphocytes. The eosinophiles have entirely disappeared. The hemaglobin remains the same.

Urine Study. A study of the urine was made after severe symptoms of trembling had developed. The following is the result:

Physical examination:
  Color—Light port wine.
  Specific gravity—1015.
Chemical examination:
  Reaction—Neutral.
  Albumin—Present.
  Sugar—None.
  Carbonates—Present.
  Sulphates—Present.
  Phosphates—Present.
  Chlorides—Present.
  Hemaglobin—None.
Microscopic examination:
  Granular tube casts—Present.
  Epithelia—
    Circular cells.
    Spindle cells.
    Squamous cells.

Albuminuria is present and the color indicates that hemaglobin may be present; however, Heller's test for the same was negative. She was normally eliminating her chlorides, carbonates, phosphates, and sulphates. Tube casts of a granular type are present.

Protocol. There is a discharge from the nostrils indicating that vomiting might have taken place. Otherwise the body presents nothing on inspection that is to be considered worthy of note. There is no conjunctivitis. The lymph glands are not palpable, and there are no cutaneous and subcutaneous lesions.

The Thoracic Cavity.—There is evidence of neither pleuritis, pneumonia, nor bronchitis. The lungs do not collapse when the thorax is opened. The sectioned surface of the lungs shows a frothy, bloody material. The lungs are congested. No ecchymoses are found on the serous surfaces of the pleura or pericardium.

The Heart.—The pericardium appears normal, and there is no evidence of endocarditis. The left side of the heart is filled with dark, coagulated blood. There is no antemortem clot. The heart muscles appear pale.

The Abdominal Cavity.—The quantity of fluid in the abdominal cavity is normal. There is no peritonitis and both small and large intestines appear normal. The abomasum and duodenum are injected and the mucosa shows evidence of muco-enteritis. No stomach worms are present.

The Spleen is normal.

The Lymph Glands are normal.

The Musculature is normal.

The Brain appears somewhat congested.

The Bladder is filled with urine.

The Liver.—The liver appears dark and congested. When cut, the blood drips from the sectioned surface.

The Kidneys.—The capsules present a metallic appearance. The kidneys appear swollen and their capsules, which are easily separable, are tense. Both active and passive congestion are noted. The glomerules can be recognized as minute red, pin-point-like spots prominent throughout the cortical portion of the kidneys. The grayish appearance of the parenchyma gives evidence of cloudy swelling.

Cultural Examination.—Two cultures on glycerine agar and plain agar from both the liver and kidney were made with negative results.

Blood smears with Romanowsky's stain were negative. Smears made from the liver substance and stained with fuchsin were likewise negative.

Microscopical Examination. *The Liver.*—Sections of the liver show advanced fatty degenerative changes. They also present the appearance of both active and passive congestion and cloudy swelling.

The Kidneys—Both active and passive congestion and glomerulitis are present. The glomerules are congested and shrunken, leaving a space between the glomerule and the capsule of Bowman (Fig. N). Cloudy swelling is present (Fig. M). In some areas the lumina of the convoluted tubules are obliterated, the cells being swollen and pushed out toward the center. Cytoplasm is granular and many of the cells have lost their nuclei.

The Lungs.—The lungs are congested and there is an occasional air cell in which are noted a few erythrocytes and polymorphonuclear leucocytes.

The Brain.—The vessels of the brain are congested. An occasional plasma cell occurs throughout the tissues. Some areas show cellular invasion, the kind of cell being mostly of the small round type. The brain cells appear normal.

The Pancreas is normal.

The Adrenal Glands are normal.

The Heart muscle shows some fatty degeneration and in these areas the muscle fibers have lost their cross-striation.

CASE 3, TABLE IX, SHOWING TEMPERATURE, PULSE, AND RESPIRATION OF LAMB 241.

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
5–11	....	....	104.1	....	....	82	......	......	74
5–12	....	....	104.0	....	....	66	......	......	70
5–13	....	104.0	....	....	63	....	......	71	......
5–14	103.1	103.0	103.2	80	84	72	78	80	48
5–15	102.9	103.1	103.0	78	120	84	57	108	114
5–16	102.5	102.6	103.2	72	72	84	84	96	108
5–17	103.7	103.9	103.0	96	88	88	168	172	150
5–18	103.7	102.2	102.5	90	84	84	150	114	118
5–19	103.6	103.2	103.6	72	70	78	156	168	190
5–20	103.5	103.0	102.7	84	84	80	180	190	186
5–21	103.1	102.5	103.8	96	90	84	186	162	168
5–22	103.4	103.7	103.6	84	78	78	178	156	178
5–23	103.9	103.7	103.3	84	90	84	129	126	156
5–24	102.8	103.2	103.2	84	78	84	120	120	108
5–25	103.0	103.0	102.4	84	78	72	126	96	144
5–26	103.4	103.8	103.7	90	87	84	102	132	120
5–27	103.3	103.2	103.7	75	84	72	96	150	156
5–28	103.8	103.3	103.5	72	84	84	144	138	132
5–29	102.8	....	102.6	84	....	84	54	......	84
5–30	102.8	103.0	103.0	72	78	84	102	114	114
5–31	103.0	103.2	103.0	84	87	75	88	62	108
6– 1	103.0	103.2	103.0	78	78	78	132	144	126
6– 2	103.1	103.4	103.0	75	81	90	138	108	132
6– 3	103.2	103.5	103.5	78	75	72	156	138	78
6– 4	103.1	104.0	103.5	75	84	87	90	86	102
6– 5	103.0	103.0	103.3	75	84	72	66	96	72
6– 6	103.4	103.6	104.0	87	75	75	126	102	108
6– 7	103.7	103.6	104.1	75	78	69	48	102	90
6– 8	104.1	104.2	103.7	78	75	78	90	102	60
6– 9	103.2	103.8	103.6	72	72	75	54	48	84
6–10	103.2	103.2	103.5	81	78	84	60	84	78
6–11	102.8	103.6	104.2	72	78	75	54	96	120*
6–12	104.9	105.2	105.4	84	84	90	102	132	154
6–13	105.6	103.7	105.7	72	84	90	120	120	126
6–14	106.2	106.2	106.0	75	78	87	144	120	66
6–15	106.3	105.4	105.7	78	72	78	60	78	66
6–16	105.9	105.9	105.6	72	81	78	54	54	60
6–17	106.0	105.4	105.4	84	78	75	96	108	78
6–18	105.9	105.1	105.4	72	90	72	66	132	96
6–19	104.8	104.6	104.4	78	81	75	72	120	72
6–20	105.0	104.8	104.4	84	72	84	66	138	102
6–21	104.2	105.1	104.8	78	78	75	66	138	108
6–22	104.4	104.7	104.4	69	72	78	54	108	60
6–23	104.3	104.7	104.6	78	78	81	114	80	80
6–24	103.6	104.3	104.0	75	84	75	60	108	126
6–25	103.8	103.7	104.6	81	72	78	102	66	132
6–26	104.2	104.5	104.8	78	75	84	102	60	108
6–27	103.4	103.8	104.0	78	75	72	96	102	84
6–28	103.2	103.6	104.2	69	66	72	36	120	126
6–29	103.8	103.4	103.9	78	75	72	96	90	60
6–30	103.7	103.3	103.5	72	54	66	54	60	42
7– 1	103.2	103.2	103.6	72	72	69	48	96	54
7– 2	103.6	103.6	103.6	78	78	66	72	84	48
7– 3	103.4	103.6	103.8	63	60	60	48	54	66
7– 4	102.9	103.3	103.5	66	72	75	42	84	54
7– 5	103.4	103.5	103.8	78	72	69	81	78	66
7– 6	103.8	103.6	103.0	78	72	69	114	66	84
7– 7	103.2	103.4	103.6	72	72	66	42	78	60
7– 8	103.3	103.4	103.4	66	66	66	60	90	72
7– 9	103.8	103.3	103.4	63	66	66	66	72	96
7–10	103.2	103.2	103.7	72	72	75	84	66	84
7–11	103.4	103.5	103.4	69	72	66	60	54	69
7–12	103.5	103.4	104.2	72	72	66	48	84	66

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*Docked.

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
7–13	104.3	103.8	103.9	66	66	72	72	72	60
7–14	103.4	103.6	104.0	66	69	75	48	48	84
7–15	103.3	103.0	103.5	66	66	60	42	78	66
7–16	103.3	103.0	103.9	66	66	66	48	54	96
7–17	103.3	103.4	103.3	66	72	72	72	90	66
7–18	102.3	103.0	102.8	66	60	66	42	48	60
7–19	103.0	103.0	102.5	60	60	57	54	48	36
7–20	102.6	102.3	103.0	63	57	54	42	48	54
7–21	102.4	102.7	103.0	60	60	60	42	54	54
7–22	103.4	103.3	102.8	66	63	66	66	42	66
7–23	103.4	103.8	103.3	66	66	63	42	54	48

This suckling lamb was unfortunately docked June 11, which accounts for the increased temperature, pulse, and respiration on that and several succeeding days.

Clinical Notes. The animal was put into the pen July 30, and was given 250 c.c. of expressed juice at 2:30 P. M. He refused supper and stood in a drooping posture. Next morning he ate about one-fourth pound of oats and was given another 250 c.c. of juice. In the evening, a third quantity of 250 c.c. of juice was given. He refused to eat in the afternoon. On August 1, breathing was accelerated and was accompanied by groaning. He refused all feed. He refused feed on the following day, gritted his teeth, breathing was jerky, and he appeared droopy. On August 3 he ate about one-fourth pound of oats and was more active, the symptoms having apparently subsided. He was given 250 c.c. of juice in the afternoon. On August 4 he refused the morning feed and 250 c.c. of juice were given. He ate all feed in the afternoon and was given another 250 c.c. of juice. On August 5 two doses of 250 c.c. each of juice were administered. A slight trembling was apparent on the morning of August 6 and he could stand without difficulty. No signs of trembling were noted in the afternoon, but he stood with head down and back bowed. He ate with normal appetite. Trembling was marked and no food was taken on August 7. In the afternoon the symptoms were more aggravated. A state of coma had set in during the following night, and on August 8 respirations were accelerated. This animal was killed on the morning of the 9th, having been in a state of coma since the night of the 7th.

Blood Study. On May 18 a blood study was made to determine the normal condition of the blood of this lamb prior to the commencement of the feeding test with white snakeroot. The following is the result:

Numerical count:	
Erythrocytes	13,440,000
Leucocytes	10,000
Differential count:	
Neutrophiles	27 per cent
Lymphocytes, large	5 per cent
Lymphocytes, small	66 per cent
Eosinophiles	1 per cent
Mast cells	1 per cent
Hemaglobin	75 per cent

On August 8 the lamb was very badly affected with trembles as a result of feeding expressed juice. The second blood study was made on this day. The results are as follows:

Numerical count:	
Erythrocytes	16,800,000
Leucocytes	7,000

Differential count:	
Neutrophiles	58 per cent
Lymphocytes, large	6 per cent
Lymphocytes, small	34 per cent
Mast cells	2 per cent
Eosinophiles	None
Hemaglobin	75 per cent

The hemaglobin remains normal and the eosinophiles disappear. There is an increase of the neutrophiles at the expense of the lymphocytes. There is a total decrease of the leucocytes and an apparent increase of the erythrocytes.

Urine Study. It was not possible to secure urine for examination before death. At autopsy a small quantity was collected through the natural passage by gentle pressure on the bladder. By the observance of this precaution, albumin from the peritoneal cavity cannot get into the urine. The result of the analysis is as follows:

Physical examination:
  Transparency—Cloudy.
  Specific gravity—1012.
Chemical examination:
  Reaction—Alkaline.
  Sugar—None.
  Albumin—Trace.
  Phosphates—Present.
  Chlorides—Present.
  Sulphates—Present.
  Carbonates—Present.

The excretion of chlorides, sulphates, and phosphates was normal and the presence of albumin alone can be regarded as an abnormality.

Protocol. The carcass presents the appearance of a normal lamb, there being no palpable lymph glands nor cutaneous and subcutaneous lesions.

The Thoracic Cavity.—The lungs are pale salmon pink in color and collapse when the thorax is opened. Neither pleuritis, pneumonia, nor bronchitis are noted. There is an excessive amount of liquid in the thoracic cavity.

The Heart—The heart is pale, flabby, and small petechiations are noted at the base along the coronary arteries. A small quantity of dark coagulated blood is found in the auricles, whereas the ventricles are empty. There is no antemortem clot.

The Abdominal Cavity.—There is no abnormal quantity of liquid in the peritoneal cavity. The intestines appear normal. There are no stomach worms in the abomasum and nodular disease and peritonitis are not present.

The Spleen is normal.

The Liver.—The liver is yellowish and when examined closely, the nutmeg appearance is found to be present. The liver is so gorged with blood that it drips from the sectioned surface. The gall bladder is full.

The Bladder contains a small quantity of cloudy urine and is slightly injected.

The Kidneys.—The kidneys have a metallic appearance. Active and passive congestion are present. The sectioned surface is pale, indicating cloudy swelling.

Microscopical Examination. *The liver* shows more or less active and passive congestion, although this condition is not so intense as in many of the other cases heretofore examined. Cloudy swelling and fatty degeneration are present.

The Kidneys.—Cloudy swelling is present. The cells of the convoluted tubules are swollen, the cytoplasm granular, and the lumina in many of the tubules are entirely obliterated. Many of the cells are in a state of necrosis. Fatty degeneration is noted in certain areas, involving the cells of the convoluted tubules. Glomerulitis is present.

The Heart.—Miescher's cysts are present. These are located in the cell substance, causing the cell wall to bulge outward. The nuclei of the heart muscle cells stain faintly. Fatty degeneration is not discernible.

The Lungs are normal.

The Pancreas is normal.

The Lymph Glands are normal.

CASE 4, TABLE X, SHOWING TEMPERATURE, PULSE, AND RESPIRATION OF LAMB 244.

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
5–11	....	....	104.0	....	....	82	......	......	78
5–12	....	....	103.5	....	....	72	......	......	66
5–13	....	103.0	....	....	79	....	......	68	......
5–14	102.8	102.6	103.4	84	84	68	78	72	70
5–15	102.6	102.7	103.2	72	84	72	70	78	120
5–16	103.0	102.9	103.0	84	84	90	96	114	156
5–17	103.0	103.0	102.9	72	84	90	132	144	168
5–18	103.5	103.3	103.3	108	96	102	168	180	186
5–19	103.5	102.3	103.5	84	90	96	178	192	216
5–20	103.9	103.1	103.5	84	90	96	192	192	204
5–21	103.3	103.1	103.5	78	90	90	168	174	174
5–22	103.6	103.3	103.6	90	90	84	150	162	210
5–23	103.8	103.5	103.6	78	84	72	114	132	144
5–24	103.6	102.8	103.2	72	72	72	156	116	132
5–25	103.4	103.0	102.9	72	84	90	108	144	144
5–26	102.9	103.2	103.0	84	84	84	54	108	132
5–27	103.4	103.1	103.3	72	90	72	120	132	138
5–28	103.4	103.4	103.1	84	87	90	114	144	108
5–29	102.8	....	103.0	72	....	84	84	......	90
5–30	103.0	102.8	102.8	84	78	84	78	102	150
5–31	102.6	103.0	103.0	78	78	72	78	96	84
6– 1	103.2	103.2	103.4	84	84	84	132	144	156
6– 2	103.2	103.2	103.5	78	72	84	126	150	136
6– 3	104.1	103.8	103.9	84	81	84	144	132	156
6– 4	103.0	103.4	103.6	87	84	78	84	102	126
6– 5	103.5	103.4	103.2	78	84	78	120	132	120
6– 6	103.7	103.7	104.0	81	75	81	102	144	150
6– 7	104.1	103.6	103.8	90	84	78	96	126	120
6– 8	104.0	104.0	104.2	81	81	78	126	132	120
6– 9	103.6	103.6	103.8	84	87	78	108	120	120
6–10	103.2	104.0	103.5	84	75	72	72	96	102
6–11	103.8	104.2	104.3	78	78	84	96	114	132*
6–12	104.2	105.0	104.6	75	78	78	126	144	192
6–13	104.8	104.7	104.8	69	72	72	192	180	192
6–14	105.4	105.6	105.1	75	69	66	192	198	168
6–15	105.6	105.5	105.8	72	66	60	150	168	168
6–16	106.0	106.4	106.5	66	72	78	120	102	126
6–17	106.4	107.0	....	60	90	....	60	78	....

---

*Docked.

These data indicate that there was a disturbance in temperature after the onset of symptoms.

Clinical Notes. This lamb was given twice daily one-fourth pound of cracked corn and oats mixed, and one-half pound of kale. She was suckling the mother, ewe 171, who received twice daily three-fourths pound of cracked corn and oats mixed and one-fourth pound of white snakeroot. In addition to this, she received, at noon, one pound of hay and two pounds of kale.

A manifest stiffness of the limbs was apparent on June 11. On June 13, she refused grain and ate only a small amount of kale. She appeared stiff, droopy, and dull. In the afternoon, respirations were slightly jerky. On the morning of June 14, she trembled slightly and was prone to lie down at all times. When made to stand she would tremble, and the longer she was kept on her feet the more intense the trembling. In the afternoon the symptoms were more aggravated. She trembled in all parts of the body and after a paroxysm of trembling would fall down apparently exhausted. At intervals she gritted her teeth. On the 15th, while lying down, she attempted to eat kale. Trembling became more aggravated, as did the gritting of teeth, and she appeared weaker than on the previous day. She did not eat during the afternoon. On the morning of June 17 she was found prostrate on her side. Respiration was labored, eyes were set, and legs were intermittently trembling. She died about 12:30 P. M.

Blood Study. On May 18 a study was made of the blood of lamb 244 to determine the normal condition, with the following results:

Numerical count:	
Erythrocytes	13,560,000
Leucocytes	12,000
Differential count:	
Neutrophiles	20 per cent
Lymphocytes, large	7 per cent
Lymphocytes, small	71 per cent
Eosinophiles	1 per cent
Mast cells	1 per cent
Hemaglobin	75 per cent

On June 16, after the symptoms of milksickness had developed, another study was made. The results are as follows:

Numerical count:	
Erythrocytes	15,600,000
Leucocytes	10,000
Differential count:	
Neutrophiles	44 per cent
Lymphocytes, large	6 per cent
Lymphocytes, small	49 per cent
Mast cells	1 per cent
Hemaglobin	75 per cent

There was an increase of neutrophiles at the expense of the lymphocytes and an apparent increase in the erythrocytes. The lamb had refused water for at least forty-eight hours before this second study was made, which may account for the apparent increase. The eosinophiles had entirely disappeared. The hemaglobin remained the same.

Urine Study. On June 17, when the autopsy was made, the urine was forced out of the normal outlet by gentle pressure upon the bladder and was thus kept free from albuminous material. The following is the result of the analysis:

Physical examination:
Sediment—Present.
Transparency—Cloudy.
Specific gravity—Insufficient amount for test.

Chemical examination:

Phosphates—Present.
Reaction—Faintly acid.
Sugar—None.
Chlorides—Present.
Albumin—None.
Carbonates—Present.
Sulphates—Present.

The urine of suckling lambs is normally acid in reaction. The cloudy condition is noted in other cases of milksickness. The lamb was apparently normally eliminating the phosphates, chlorides, and sulphates.

Protocol. There are no subcutaneous nor cutaneous lesions to be found. There are no palpable lymph glands, and the external appearance is that of a normal lamb.

Thoracic Cavity.—The lungs do not collapse when the thorax is opened. There is present a congestive condition, but neither pneumonia, bronchopneumonia, nor pleuritis. The lungs are dark pink in color.

The Heart.—Petechiation at the the base of the heart along the course of the coronary arteries is noted. The right side of the heart is empty, while the left contains dark coagulated blood. There are no petechiations on the endocardium or pericardium.

The Abdominal Cavity.—There is no abnormal quantity of fluid in the abdominal cavity. There is no peritonitis. The small and large intestines are affected with nodular disease and stomach worms are present in small numbers in the abomasum. The small intestine appears injected, which condition does not exist in the large intestine.

The Spleen.—Normal.

The Kidneys.—The kidneys appear swollen and of a metallic appearance. The capsule strips off easily. Both active and passive congestion and cloudy swelling are present. Both kidneys weigh one-eighth pound.

The Musculature.—The musculature appears normal.

The Liver.—The liver is filled with blood which drips from the sectioned surface. It is nutmeg in appearance. There is evidence both of congestion and of cloudy swelling. The liver weighs one-fourth pound.

The Lymph Glands.—The lymph glands appear normal.

The Brain.—The brain appears somewhat congested.

Microscopical Examination. *The Liver.*—After hardening in formalin, sectioning, staining with hematoxylon and eosin, and clarifying in beechwood creosote, study was made of the cellular changes.

The liver shows both active and passive congestion and cloudy swelling.

The Kidneys.—Active and passive congestion are present. There is an occasional area infiltrated with polymorphonuclear leucocytes and with a few round cells. Cloudy swelling in various stages is present, thus indicating an acute parenchymatous nephritis.

The Heart.—The blood vessels are congested. There is present no fatty degeneration.

The Brain.—The cerebrum and cerebellum are both congested. The brain cells appear normal with no invasion by leucocytes.

The Lungs.—The lung tissue shows congestion, certain alveoli are filled with serum and an occasional one with a few erythrocytes.

The Pancreas—Normal.

The Spleen.—Normal.

The Adrenal Glands.—Normal.

CASE 5, TABLE XI, SHOWING TEMPERATURE, PULSE, AND RESPIRATION OF EWE 12.

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
5–11	....	....	103.3	....	....	68	......	......	72
5–12	....	....	104.0	....	....	65	......	......	60
5–13	....	101.4	....	....	56	....	......	48	......
5–14	101.0	101.2	102.2	58	66	48	52	45	30
5–15	100.5	101.2	101.9	60	54	66	42	42	42
5–16	101.3	102.0	102.1	56	70	66	48	48	54
5–17	101.5	102.0	103.0	66	66	66	36	42	54
5–18	101.4	101.8	102.6	72	76	72	48	54	72
5–19	101.8	101.9	102.7	60	63	72	48	48	78
5–20	102.0	102.0	101.9	66	72	72	42	45	45
5–21	101.9	102.2	103.2	69	66	72	39	48	90
5–22	102.0	101.9	102.9	72	60	69	36	42	72
5–23	101.8	101.6	102.4	57	66	60	33	48	42
5–24	101.5	101.6	102.2	66	60	63	30	36	36
5–25	101.6	101.4	102.3	54	63	66	30	36	36
5–26	102.0	102.2	103.0	63	66	69	30	27	35
5–27	102.0	102.3	103.2	72	72	75	30	42	84
5–28	102.3	102.2	103.4	72	63	72	30	48	48
5–29	101.8	....	102.6	66	....	66	42	......	36
5–30	101.5	101.5	102.3	69	60	69	42	38	42
5–31	101.2	101.6	102.4	69	72	60	39	48	36
6– 1	101.7	102.6	103.6	69	66	78	36	54	84
6– 2	103.0	102.5	103.0	66	72	78	33	48	66
6– 3	103.1	102.8	102.8	66	66	69	54	51	60
6– 4	102.2	102.4	102.6	60	66	66	36	36	30
6– 5	102.3	102.5	103.8	66	66	72	36	23	48
6– 6	102.0	102.0	103.3	72	66	66	42	39	84
6– 7	102.3	102.6	103.8	60	66	63	36	42	54
6– 8	102.2	102.6	103.4	69	66	66	36	42	30
6– 9	101.8	102.2	102.0	60	66	69	30	36	36
6–10	102.0	102.0	102.7	66	66	60	27	30	27
6–11	101.5	101.8	103.7	72	69	66	30	27	48
6–12	101.6	102.6	103.5	69	60	66	36	48	72
6–13	102.2	103.0	103.4	63	72	68	36	66	54
6–14	102.4	102.0	103.1	72	72	66	30	75	30
6–15	102.2	102.8	103.4	72	69	72	42	36	48
6–16	102.7	102.4	103.0	69	69	72	30	30	42
6–17	102.2	102.2	103.0	72	66	63	42	66	42
6–18	102.0	102.0	104.0	66	72	66	42	36	48
6–19	102.6	102.6	102.8	69	75	66	39	84	60
6–20	102.7	102.7	103.0	66	66	69	45	60	57
6–21	102.2	103.2	103.4	66	72	66	54	60	72
6–22	102.7	102.5	102.7	72	66	66	39	48	54
6–23	102.8	103.3	103.4	66	72	69	63	78	72
6–24	102.0	102.4	103.8	72	66	78	54	72	120
6–25	102.8	103.0	103.2	63	66	66	42	72	72
6–26	102.6	102.5	103.7	60	72	72	42	60	102
6–27	102.8	102.6	103.2	72	69	69	48	72	84
6–28	102.6	102.6	103.6	66	60	69	42	54	78
6–29	102.7	102.6	102.8	69	69	72	48	54	36
6–30	102.4	102.0	103.2	69	63	69	36	54	72
7– 1	103.0	102.7	102.4	66	66	69	48	54	72
7– 2	102.6	102.5	103.0	75	72	66	36	60	48
7– 3	102.5	102.6	103.3	72	69	66	42	54	72
7– 4	101.6	102.4	102.5	72	72	69	42	63	74
7– 5	102.1	103.0	103.3	66	72	69	36	60	54
7– 6	102.6	102.8	103.0	72	69	63	42	66	54
7– 7	102.6	102.6	102.8	60	69	66	48	54	42
7– 8	102.3	103.0	102.3	66	72	69	54	60	72
7– 9	102.6	103.0	103.3	72	66	72	54	60	96
7–10	102.8	102.9	103.4	72	69	69	54	66	72
7–11	102.6	103.2	103.7	72	66	72	48	78	66
7–12	103.4	103.3	103.4	63	69	66	48	66	90
7–13	102.3	102.9	103.4	66	66	72	54	54	66
7–14	103.0	103.1	103.5	72	66	69	42	66	78
7–15	103.0	103.0	103.2	72	66	63	36	69	66
7–16	102.7	102.6	103.2	66	66	66	48	60	108
7–17	102.6	103.0	103.2	72	72	69	36	90	72
7–18	101.5	102.5	102.7	66	60	60	48	42	54
7–19	101.5	102.2	103.5	69	66	84	72	78	60
7–20	101.3	101.7	102.7	69	78	78	66	54	69
7–21	101.1	101.9	101.9	78	72	75	90	84	84
7–22	102.0	102.1	102.4	72	81	78	60	72	63
7–23	102.5	102.3	102.3	72	69	66	60	60	54

These data do not show any variation from the normal in temperature, pulse, or respiration.

Clinical Notes. On July 17 ewe 12 appeared droopy, dull, and stood with eyes half-closed. On the 19th breathing was jerky and trembling was noted in both fore and hind limbs. After she had lain quiet for a while she would stand for several minutes, and with the onset of trembling would lie down again. Her appetite was good. On July 21 the symptoms were more aggravated. She would stand with all four feet close together under the body, with back arched, and was quite stiff. On the 22d, trembling was more violent, but her appetite remained good. In the afternoon, she passed into a comatose condition. There was little appreciable change on July 23, but the symptoms were more aggravated. On July 24 she was killed and an autopsy was performed.

It should be noted with reference to this ewe that she had recovered from trembles from feeding experiments of the previous year. This indicates that one attack does not render immunity to a subsequent attack, a fact in keeping with popular belief.

Blood Study. On May 18 a study was made of the blood of ewe 12 to determine its normal condition. The following is the result:

Numerical count:	
Erythrocytes	8,240,000
Leucocytes	12,000
Differential count:	
Neutrophiles	47 per cent
Lymphocytes, large	7 per cent
Lymphocytes, small	40 per cent
Eosinophiles	3 per cent
Mast cells	None
Hemaglobin	70 per cent

On July 22, after symptoms of trembles had become well developed, another blood study was made with the following result:

Numerical count:	
Erythrocytes	8,320,000
Leucocytes	5,000
Differential count:	
Neutrophiles	66 per cent
Lymphocytes, large	8 per cent
Lymphocytes, small	22 per cent
Eosinophiles	None
Mast cells	4 per cent
Hemaglobin	75 per cent

There was an increase of neutrophiles at the expense of the small lymphocytes. There was a disappearance of the eosinophiles and the appearance of mast cells. The hemaglobin remained practically the same and there was no change in the number of erythrocytes. There was a considerable decrease in the leucocytes.

Urine Study. An analysis of the urine was made on June 18, with the following results:

Physical examination:
Transparency—Pale straw color.
Specific gravity—1014.

Chemical examination:
Reaction—Alkaline.
Sugar—Present.
Albumin—Present.
Phosphates—Present.
Carbonates—Present.
Chlorides—Present.
Sulphates—Present.

On July 19, after the symptoms of trembles were well developed, the urine was again analyzed. The following is the result of this analysis:

Physical examination:
Transparency—Brownish yellow.
Sediment—Present.
Specific gravity—1030.

Chemical examination:
Reaction—Neutral.
Sugar—Present.
Albumin—Present.
Carbonates—Present.
Sulphates—Present.
Chlorides—Present.
Phosphates—Present.
Microscopic examination:
Tube casts—Present.
Character of casts—Granular (Fig. O).
Epithelial cells—Round, squamous, elliptical.

Protocol. Externally the body presents nothing on inspection that is to be considered worthy of note. There are no subcutaneous lesions of any kind to be found.

Thoracic Cavity.—There is no pleuritis. The lungs do not collapse when the thorax is opened. The color of the lungs is a purplish-salmon. No edema, pneumonia, nor broncho-pneumonia are present. No ecchymoses are found on the pleural surface.

The Heart.—There is slightly more fluid than is normal in the pericardial sac. Small petechial areas occur over the base of the heart. There is no pericarditis nor endocarditis. The right side of the heart is empty and the left side contains dark, coagulated blood, post mortem coagulation. The heart muscles appear pale.

The Abdominal Cavity.—There is no abnormal quantity of fluid in the abdominal cavity. The small and large intestines are affected with nodular disease. Few stomach worms are to be found in the abomasum. The small intestine is highly injected and the large intestines appear normal in this respect.

The Spleen.—Normal.

The Kidneys.—The kidneys appear swollen, the capsule having a metallic appearance. Both active and passive congestion are discernible. Cloudy swelling is present. Both kidneys weigh four and one-half ounces.

The Liver.—The liver shows the characteristic discoloration and blood flows freely from the sectioned surface.

The bladder.—The bladder is injected and contains a small quantity of urine.

Musculature.—The musculature is normal in appearance.

The Brain.—The brain, as well as the coverings, appear congested.

The Lymph Glands.—All visceral and body lymph glands appear normal.

Microscopical Examination. *The Liver.*—The tissues were first hardened in formaldehyde, sections were then made, which were stained in hematoxylon and eosin, and clarified in beechwood creosote.

The liver shows both active and passive congestion, cloudy swelling and fatty degeneration.

The Kidneys.—The kidneys show both active and passive congestion. Cloudy swelling and glomerulitis are present. Some of the cells of the convoluted tubules have lost their nuclei and are in a state of necrosis. In some areas the lumina are not discernible and the bases of the cells have separated, the whole mass pushing toward the center. Some of the tubules show evidence of fatty degeneration.

The Brain.—The blood vessels are congested. An occasional polymorphonuclear leucocyte and quite a number of plasma cells are in each microscopic field. The brain cells appear normal and are not shrunken.

The Heart.—The heart muscle fibers have lost their cross-striation. Many parts show fatty degeneration. The nuclei stain deeply, in fact, are picnotic. The blood vessels are congested.

The Lungs.—The lungs are hyperemic. Some alveoli are filled with serum. There is no bronchitis nor pneumonia.

The Duodenum.—Much round-celled infiltration and hyperemia are evident.

The Pancreas.—Normal.

The Adrenal Gland.—Normal.

CASE 6, TABLE XII, SHOWING TEMPERATURE, PULSE, AND RESPIRATION OF LAMB 237.

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
5–11	....	....	104.6	....	....	70	......	......	72
5–12	....	....	103.6	....	....	72	......	......	72
5–13	....	103.8	....	....	76	....	......	84	......
5–14	102.7	101.8	103.0	78	78	48	60	72	60
5–15	104.2	104.1	103.5	84	90	72	84	108	108
5–16	102.1	102.7	103.1	84	72	78	72	78	96
5–17	102.3	102.3	103.0	78	80	84	90	102	144
5–18	102.6	103.0	102.7	78	80	78	144	156	150
5–19	102.9	102.6	102.9	80	84	78	178	190	198
5–20	102.5	102.1	102.5	90	90	84	180	186	182
5–21	103.1	103.2	103.4	78	78	84	193	163	144
5–22	102.6	102.8	103.5	78	90	84	138	162	156
5–23	103.2	103.0	103.0	90	84	78	126	144	120
5–24	102.8	102.6	103.2	78	84	78	84	102	96
5–25	103.0	103.2	103.0	72	75	78	108	108	108
5–26	103.0	102.8	103.2	84	75	84	90	102	102
5–27	103.4	103.0	103.2	75	75	78	84	114	108
5–28	103.4	102.9	103.0	72	66	72	102	78	72
5–29	103.0	....	102.9	72	....	84	66	......	60
5–30	103.2	103.0	103.1	72	69	75	60	108	120
5–31	102.6	102.5	102.8	78	84	78	72	78	102
6– 1	103.4	103.1	103.8	84	78	78	120	147	144
6– 2	102.8	103.0	103.0	72	84	72	126	132	132
6– 3	103.5	103.7	103.6	81	78	84	156	138	120
6– 4	102.8	103.0	103.2	72	84	72	66	69	96
6– 5	103.2	103.0	103.2	81	72	75	102	120	114
6– 6	103.4	103.2	103.6	72	81	75	102	108	108
6– 7	103.6	103.0	104.2	72	78	84	108	96	120
6– 8	103.4	103.2	103.6	75	72	72	108	120	108
6– 9	103.0	103.6	103.5	72	72	75	84	108	84

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
6–10	103.4	103.8	103.7	72	72	75	48	75	54
6–11	103.2	103.0	104.5	78	78	81	48	84	108
6–12	103.8	103.5	103.8	84	75	72	126	120	132
6–13	104.1	104.0	104.2	75	78	72	120	126	144
6–14	104.0	104.0	103.3	78	75	75	108	120	90
6–15	103.6	103.5	104.0	78	78	78	66	60	84
6–16	103.0	103.8	104.0	78	78	72	60	54	60
6–17	103.4	103.3	104.1	78	72	72	66	78	72
6–18	102.8	103.4	104.1	75	75	72	78	102	96
6–19	103.6	103.7	104.0	78	78	75	84	90	114
6–20	103.4	103.9	104.2	72	72	72	90	84	96
6–21	104.0	104.1	104.4	78	75	72	84	96	102
6–22	103.7	103.5	103.8	72	72	75	78	108	96
6–23	104.4	103.3	104.4	78	75	78	84	108	104
6–24	104.0	104.2	104.6	78	75	75	90	120	144
6–25	104.1	104.2	104.4	66	75	72	126	126	144
6–26	103.6	103.9	104.6	69	72	81	78	120	120
6–27	104.6	104.5	105.0	78	72	72	120	138	126
6–28	104.6	104.4	104.0	78	78	72	90	108	114
6–29	104.0	104.1	104.0	75	72	72	90	102	66
6–30	103.8	103.4	104.0	72	72	78	72	96	102
7– 1	103.7	104.0	104.2	69	69	72	78	108	78
7– 2	104.2	103.8	103.0	72	75	72	90	99	78
7– 3	103.2	103.7	103.4	69	66	54	60	66	66
7– 4	103.0	103.6	103.8	72	69	60	66	90	90
7– 5	103.9	103.5	103.8	81	72	66	90	96	120
7– 6	104.2	103.6	103.4	72	66	72	78	60	84
7– 7	103.6	103.6	103.7	69	66	72	66	42	72
7– 8	103.9	103.3	103.6	60	69	60	48	84	72
7– 9	104.0	103.6	104.2	66	69	66	90	96	90
7–10	103.3	103.8	104.7	66	63	72	60	102	120
7–11	103.8	103.8	104.0	69	66	78	72	54	66
7–12	103.8	103.7	104.1	60	66	69	72	96	78
7–13	103.8	103.5	103.8	72	66	69	51	72	84
7–14	103.8	103.5	103.8	66	66	72	48	102	114
7–15	104.1	103.9	104.2	66	66	72	60	90	96
7–16	104.1	103.9	104.2	63	60	66	54	60	96
7–17	104.0	104.0	103.8	66	69	66	96	96	72
7–18	103.5	103.4	103.6	60	63	66	54	60	66
7–19	103.2	103.8	104.2	63	66	57	48	54	48
7–20	103.0	103.7	103.1	66	66	60	72	57	42
7–21	102.7	103.0	103.8	57	60	63	48	51	54
7–22	104.1	104.3	103.7	69	66	66	60	84	84
7–23	104.0	104.1	104.5	60	60	60	66	84	96
7–24	104.0	104.4	104.1	66	69	66	78	114	84

Clinical Notes. On July 30 at 2:30 P. M. lamb 237 was taken from the pasture, placed in the experimental pen and given 500 c.c. of expressed juice. He refused his feed in the evening. Next morning breathing was short and labored and he gritted his teeth. He ate about one-fourth pound of oats and was given 500 c.c. more of juice. At 3 P. M. a third quantity of 500 c.c. was given. He refused his feed in the evening. On the morning of August 1 he was found dead.

Protocol. The external appearance of the carcass is that of a normal lamb.

The Thoracic Cavity.—Neither pleuritis nor pneumonia is present. The lungs are a pale salmon color and collapse when the thoracic cavity is opened.

The Heart.—There is no excessive fluid in the pericardial sac. A small amount of coagulated dark blood is found in the left side, the right being empty. The heart muscle appears normal.

The Abdominal Cavity.—The abdominal cavity does not contain an excessive amount of fluid and there is no peritonitis present. The bladder is empty and appears normal. The stomach and small and large intestines appear normal. Neither nodular disease nor stomach worms are present.

The Spleen.—Normal.

The Musculature.—Normal.

The Brain.—Normal.

The Lymph Glands—Normal.

The Liver.—The liver appears normal in color and size. The gall bladder is full.

The Kidneys.—The capsule has a metallic appearance and can easily be stripped off. Both active and passive congestion are evidenced. The grayish appearance of the parenchyma also gives evidence of cloudy swelling.

Microscopical Examination. *The Liver.*—Active and passive congestion and cloudy swelling are present.

The Kidneys.—Glomerulitis and cloudy swellng are present. In some areas the nuclei take little or no stain and in others the cells have lost their nuclei. The lumina in these areas are occluded and many cells are thus in a state of necrosis. Some cells are in a state of picnosis. Certain of the tubules show fatty degeneration.

The Pancreas.—Normal.

The Adrenal Glands.—Normal.

The Lungs.—Normal.

The Heart.—Normal.

CASE 7, TABLE XIII, SHOWING TEMPERATURE, PULSE, AND RESPIRATION OF LAMB 242.

	Temperature.			*Pulse.*			*Respiration.*		
Date.	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*	*A.M.*	*M.*	*P.M.*
5–11	....	....	104.6	....	....	67	......	......	42
5–12	....	....	104.0	....	....	66	......	......	72
5–13	....	103.0	....	....	84	....	......	72	......
5–14	102.8	102.4	102.7	76	80	66	72	78	42
5–15	102.6	102.9	103.1	63	78	78	60	66	96
5–16	102.3	102.6	103.1	60	72	96	48	63	90
5–17	103.1	102.6	102.6	78	96	86	78	114	120
5–18	103.1	103.0	103.0	96	96	96	138	172	180
5–19	103.6	103.6	103.1	78	84	90	150	160	192
5–20	103.0	102.7	102.8	78	90	90	144	150	163
5–21	103.4	102.8	103.3	84	84	96	168	144	168
5–22	103.6	102.8	103.4	84	84	72	156	150	162
5–23	103.4	103.2	103.5	78	90	84	120	138	144
5–24	103.0	102.8	103.0	78	72	84	114	102	108
5–25	103.0	103.0	103.2	78	71	87	120	138	132
5–26	103.0	108.8	103.8	78	90	90	90	120	150
5–27	103.0	102.4	103.2	84	90	87	123	152	150
5–28	103.2	103.2	103.1	90	75	84	138	126	108
5–29	103.0	....	102.9	84	....	84	96	......	90
5–30	102.7	102.6	103.3	90	84	84	96	102	108
5–31	102.8	103.0	103.0	90	78	78	72	96	114
6– 1	103.3	103.4	103.4	69	78	90	102	144	150
6– 2	103.1	103.0	103.9	81	78	90	126	132	114
6– 3	103.3	103.0	103.9	81	90	87	138	120	168
6– 4	102.7	103.0	103.7	78	84	84	84	96	102
6– 5	103.2	102.9	102.8	78	78	75	78	132	102
6– 6	103.5	103.7	104.0	87	72	87	132	150	162
6– 7	103.9	103.6	104.2	84	78	81	126	120	144
6– 8	104.2	103.8	103.8	81	87	90	132	138	124
6– 9	103.6	103.2	103.9	84	78	84	108	120	132
6–10	103.4	103.6	103.5	81	84	72	72	96	78
6–11	103.3	103.5	104.2	81	84	78	90	126	138*
6–12	104.2	104.2	105.1	78	78	72	150	156	144
6–13	104.1	104.7	105.3	78	84	84	132	150	162
6–14	105.8	105.6	105.4	72	72	84	192	180	132
6–15	105.0	105.0	105.1	69	72	66	60	114	138
6–16	105.9	106.2	Died	66	66	....	108	60	......

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*Docked.

Clinical Notes. On the morning of June 13 there was a manifest stiffness of the legs. The next day the lamb stood with head down and back bowed. On the 15th she did not touch the kale or grain and upon being exercised began to tremble. She did not remain long on her feet, but lay down as though exhausted. Trembling was aggravated with the additional symptoms of vomiting, frothing at the mouth, grating of the teeth, and labored breathing on the 16th. She died at 1:30 P. M.

Blood Study. On May 18 a study was made of the blood to determine the normal condition of this lamb's blood with the following result:

Numerical count:	
Erythrocytes	13,040,000
Leucocytes	14,000
Differential count:	
Neutrophiles	20 per cent
Lymphocytes, large	16 per cent
Lymphocytes, small	61 per cent
Eosinophiles	3 per cent

On June 15, after the symptoms had developed, another study of the blood was made with the following results:

Numerical count:	
Erythrocytes	14,000,000
Leucocytes	11,000
Differential count:	
Neutrophiles	46 per cent
Lymphocytes, large	2 per cent
Lymphocytes, small	52 per cent
Eosinophiles	None

This shows an increase of neutrophiles with a corresponding decrease of lymphocytes. The eosinophiles have disappeared. There is a decrease in the total number of leucocytes, and apparently a slight increase of erythrocytes.

Protocol. There are no cutaneous or subcutaneous lesions to be found. The lymph glands are not palpable and the external appearance is that of a normal lamb.

The Thoracic Cavity.—The lungs are of pale salmon color and collapse when the thorax is opened. There is present neither pneumonia nor pleuritis.

The Heart.—The heart weighs one-fourth pound. It shows small petechiæ along the course of the coronary arteries. The heart muscle appears normal.

The Abdominal Cavity.—The quantity of fluid in the abdominal cavity is normal and there is no peritonitis. The small and large intestines appear normal.

The Spleen.—Normal.

The Musculature.—Normal.

The Kidneys.—The kidneys are very dark in color and the surface appears slightly mottled, due to intense congestion. Both active and passive congestion are present. The sectioned surface is grayish, indicating cloudy swelling. Both kidneys weigh one-eighth pound. The capsule strips off easily.

The Adrenal Glands.—Normal.

The Lymph Glands.—Normal.

The Liver.—The liver is normal in color. It weighs one and one-eighth pounds. The gall bladder is full.

The Brain.—The coverings and brain substance are normal in appearance.

Microscopical Examination. *The Liver.*—Fatty degeneration, active and passive congestion and cloudy swelling are present.

The Kidneys.—Active and passive congestion are present. The nuclei of the parenchymatous cells, in some areas, are picnotic; in others, they stain faintly. The protoplasm is granular and the cells swollen. The lumina of certain tubules are partially occluded and in some places the lumina of the convoluted tubules have disappeared. The nuclei, especially of these convoluted tubules, have entirely disappeared and the cells are in a state of necrosis. Many of the glomerules appear shrunken and a wide space can be noted between the capillary plexus and the capsule of Bowman. Thus acute parenchymatous nephritis with glomerulitis and active and passive congestion are present.

The Heart.—The blood vessels are congested.

The Brain.—The brain is congested. This congestion involves both arteries and veins. The spinal cord also shows congestion.

The Lungs.—Normal.

The Pancreas.—Normal.

The Adrenal Gland.—Normal.

The following microscopic studies were made of the livers and kidneys of six guinea pigs:

Microscopical Examination. Case 1.—Acid extract, No. 1.

Liver.—Congestion, fatty degeneration, and cloudy swelling are marked. Congestion, both active and passive, is more intense than in sheep.

Kidneys.—The changes in the kidneys include active and passive congestion, fatty degeneration, and cloudy swelling.

Case 2.—Green stems, No. 2.

Liver.—Cloudy swelling, fatty degeneration, and active and passive congestion are all present. An occasional area is invaded by polymorphonuclear leucocytes.

Kidneys.—The metamorphoses include active and passive congestion, cloudy swelling, and glomerulitis.

Case 3.—Green leaves.

Kidneys.—Glomerulitis, active and passive congestion, and cloudy swelling are very pronounced.

Liver.—Active and passive congestion and cloudy swelling are present. There are fatty degenerative changes in some areas.

Case 4.—Expressed juice, No. 1.

Liver.—Active and passive congestion and cloudy swelling are apparent. There are, furthermore, areas showing the beginnings of fatty degeneration.

Kidney.—The pathological changes include active and passive congestion, cloudy swelling, and glomerulitis.

Cultural studies were made of the blood from the liver, kidneys, and heart. Three tubes of agar were inoculated and all except one were negative. In this tube, a polar staining, short bacterium was isolated in pure culture. Intraperitoneal inoculation on guinea pigs resulted in no evidence of disease. The germ in question was, therefore, considered as a contamination.

CASE 5.—Expressed juice, No. 2.

Liver.—Cloudy swelling and fatty degeneration are present. This liver does not show active or passive congestion.

Kidneys.—Active and passive congestion are present. Cloudy swelling is well advanced in the convoluted tubules, in many of which the cells are in a state of disintegration.

CASE 6.—Green leaves, No. 2.

Liver.—Fatty degeneration was more advanced than in Case 4. Active and passive congestion are present.

Kidneys.—Active and passive congestion and cloudy swelling are seen in the renal tissues. Some of the convoluted tubules show fatty degeneration.

The following report covers the clinical examination of one hog which died during the feeding experiments:

Urine Study. An analysis of the urine shows the reaction to be acid. The specific gravity is 1014. Tests for albumin and sugar were negative.

Protocol. There are no subcutaneous or cutaneous lesions to be found, and no palpable lymph glands. The external appearance presents the picture of a young hog in a poor condition of flesh. The conjunctiva are pale.

Thoracic Cavity.—The lungs do not collapse when the thorax is opened, and certain of the lobules show congestion. When the sectioned surface of the lungs is examined, the bronchi are found to contain a frothy material.

The Heart.—The heart muscle appears pale, but there is no petechiation. There is present a slight evidence of a hydropericardial condition, and the pleura is normal.

The Abdominal Cavity.—The peritoneum, spleen and lymph glands are normal. The liver presents a pale, parboiled appearance, the lobules show very distinctly, and from the sectioned surface can be scraped a material which has the appearance of fat, and ignites when held in a flame. Duodenitis is present.

The Kidneys.—The kidneys appear normal in size and are of the same parboiled appearance as was noted in the case of the liver.

The Bladder.—Filled with urine.

The Musculature.—The general musculature of the body appears paler than normal.

Microscopical Examination. *The Liver.*—The liver shows both cloudy swelling and fatty degeneration in rather advanced stages.

The Kidneys.—Fatty degeneration is evident as are also various stages of cloudy swelling.

The Heart.—The nuclei stain pale, but the muscle cells have not lost their cross-striation.

The Brain.—The brain, both cerebrum and cerebellum, appear normal.

The Lungs.—Some lobules show edema and congestion.

The Pancreas.—Normal.

The Spleen.—Normal.

The Adrenal Glands.—Normal.

Cultural Examination. No growth appeared in attempted isolations of organisms from the blood of the kidney and liver of this animal.

SUMMARY OF LESIONS

The following is a tabulation of the lesions of sheep and lambs dead of trembles and milksickness, from a total of 16 cases:

Gross	*No.*	*Per cent*
Petechia, epicardium	13	81
Pale heart muscle	3	18
Muco-enteritis, small intestine	12	75
Active and passive congestion, kidneys	15	93
Cloudy swelling, kidneys	15	93
Congestion of brain and meninges	6	37
Lungs do not collapse, slight edema	5	31
Congestion, liver	12	75
Cloudy swelling, liver	12	75
Hydropericardium*	4	25

The following is a tabulation of the microscopic study of the organs of 16 sheep and lambs dead of trembles:

Microscopic		
Liver:	*No.*	*Per cent*
Active congestion	9	56
Passive congestion	8	50
Cloudy swelling	13	81
Fatty degeneration	14	87
Kidneys:		
Active congestion	8	50
Passive congestion	7	43
Cloudy swelling	16	100
Glomerulitis	9	56
Fatty degeneration	5	31
Brain:		
Congestion†	8	50
Heart:		
Fatty degeneration	5	31
Congestion	4	25
Lungs:		
Hyperemia	5	31
Edema	4	25
Duodenum:		
Muco-enteritis	12	75

The most striking pathological features in trembles or milksickness are therefore the albuminoid and fatty metamorphoses of the parenchymatous organs.

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*These sheep were infested with Hemonchus contortus, which may account for any hydropericardium present.

†Only twelve cases were studied microscopically.

Cultures were made from livers, hearts, and kidneys of a total of six sheep and lambs and one hog dead of the disease, all of which cultures were negative. Cultures were made from the heart, liver and kidneys of a guinea pig dead of the disease, one of which cultures showed a growth, which organism later proved to be a contamination.

NATURE OF THE POISONOUS PRINCIPLE

Since a relatively recent claim has been made (Moseley, 1909) that the feeding of aluminium phosphate is followed by effects similar to those following the feeding of white snakeroot, an attempt was made in 1916 to produce trembles by feeding aluminium phosphate to sheep. Two ewes, 166 and 167, were accordingly maintained on this experiment from September 9 to November 17. During this time each ewe was fed 412 gm. of aluminium phosphate ($Al\ PO_4$; Baker's C. P.) mixed with 68.5 pounds of grain, this being supplemented with 138 pounds of alfalfa hay. The daily amounts of aluminium phosphate given were gradually increased from 2 to 16 gm.

At no time during this period of 69 days were these ewes observed to manifest any symptoms of trembles. The initial weight of ewe 166 was 80 pounds, and her weight at the close of the experiment was 91 pounds. The initial and final weights of ewe 167 were 90 and 93 pounds, respectively.

These results indicate that aluminium phosphate is not the active principle in white snakeroot and are in accord with statements more recently made by Moseley (1917).

Certain of the experiments with sheep and guinea pigs which were made in 1917 indicate a number of points with reference to the nature of the active substance in the production of trembles and milksickness. No evidence has been obtained that it is water soluble, or alcohol soluble, and evidence of its solubility in dilute acid is inconclusive. With long desiccation at a temperature of about 100 degrees C., the substance seems to lose its toxicity. Such is not the case when the expressed juice is evaporated at about 60 degrees C. The plant furthermore contains a nontoxic volatile oil which does not pass into the milk; at least it has not been possible in the several attempts which have been made to distil it from milk from a cow fed on white snakeroot. There is furthermore present a considerable amount of resinous material which, according to a recent account by Moseley (1917), exists in the leaves to the extent of 9 per cent of their dry weight and is the poisonous principle. This claim, however, is not supported by conclusive experimental data.

A number of the more common reagents for alkaloids were employed on the presumption that the plant contains an alkaloid, which is the active principle. No precipitate appeared when Mayer's reagent was added to expressed sap. Potassium ter-iodide, phosphotungstic acid, auric chlorid and platinic chlorid failed to yield precipitates. With a saturated solution of tannic acid, however, a copious precipitate appeared. Since many organic substances are precipitated with tannic acid, and since negative results were secured with all of the other common precipitants, there is little likelihood of the presence of an active alkaloid in white snakeroot.

Since the glucosides, eupatorin and euparin, are known to occur in Eupatorium perfoliatum and E. purpureum respectively, two species commonly present within the State, and since certain glucosides are known to possess toxicological properties, Bourqelot's general group test for the presence of reducing sugars after hydrolysis was made. A quantity of the expressed sap of the plant was made acid by the addition of 1 per cent by volume of concentrated hydrochloric acid and permitted to stand for twenty hours before analysis. Another portion was kept for an equal period of time at about 30 degrees C. to favor activity by enzymes within the sap. A third quantity was freshly expressed when needed for analysis. One hundred cubic centimeters of each sample were diluted with 400 c.c. of water, clarified with alumina cream and an aliquot, then taken for analysis. The official method* for the estimation of reducing sugars was followed. The results of this analysis showed the presence of approximately .10 gm. of reducing sugar in 100 c.c. of freshly prepared sap, of .13 gm. in 100 c.c. when enzymes within the expressed sap were permitted to act, and of .20 gm. in 100 c.c. when dilute acid was used. Hydrolysis, therefore, resulted from enzymotic activity and from the use of weak acid. This may be taken to indicate the presence of glucosides in the sap of white snakeroot. It is realized, of course, that there may occur within plants substances which can reduce Fehling's solution without themselves undergoing preliminary hydrolysis, and that substances other than glucosides yield glucose on hydrolysis. Further studies on the nature of the active principle are in progress.

No particular difficulty need be encountered in explaining the excretion of this poisonous principle in the milk, a fact which has long been supported by more or less experimental evidence prior to the present studies. It is a matter of common knowledge that milk acquires tastes and flavors from the feed of the animal. This in itself is proof that foreign substances find entrance into the milk. Furthermore, the medical practitioner well knows that such substances as opium, morphine, and atropin may pass into the mother's milk and act on the nursing child. Not only do organic substances pass into the milk, but many inorganic substances as well,‡ as arsenic, iodine, lead, zinc, mercury, iron, bismuth, and antimony.

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*Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 42, 1912.

MEANS OF PREVENTION AND CURE

Prophylactic or preventive measures, rather than curative measures, are to be employed in dealing with trembles and milksickness, particularly in the case of domestic animals. In districts where the disease was prevalent, early settlers found, by keeping their animals from pasturing upon certain lands or by cutting down the timber and exposing the land to sunlight, that they could avoid the disease. They furthermore found that by plowing dangerous areas and seeding them to cultivated forage plants, they could render them safe for their stock. The employment of either of these preventive measures is in some localities practicable under North Carolina conditions. Where the timbered lands are under the control of timber companies it is not feasible to clear them, and furthermore it would in many places not be advisable to do so because of the value of the timber. If it is possible to clear the land, it should be done since white snakeroot is intolerant to full sunlight. Areas occupied by this weed might in some cases be fenced off, some of them might be mown several times during a growing season, or might even be plowed up. Since the weed is perennial rooted, a single mowing off of the tops, or even a single plowing, would not destroy all of the plants. The destruction of at most a few acres of white snakeroot will in many large pastures render an otherwise dangerous pasture harmless.

Various forms of treatment have been suggested for animals suffering from the disease, but the nature of the changes within the internal organs, as has been described, are such as to extend little hope that beneficial results can follow medication. Among the classes of drugs which veterinarians employ are cathartics such as eserin, internal antiseptics, and nerve stimulants, but without consistent remedial effects. Large doses of sodium bicarbonate are relied upon by others to effect a cure.

Our own experimental work on this phase of the problem has been confined to the use of common salt and baking soda, since the accounts of Moseley (1910) and others suggest that sodium compounds have antidotal effects. Three animals were employed in this experiment, which was conducted between September 18 and October 20, 1916. The essential facts in this experiment are assembled in Table XIV.

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‡ *Vide*, Hammarsten and Hedin, a text-book of Physiological Chemistry, 7th edit., 1914, p. 671.

TABLE XIV.—RESULTS OF FEEDING WHITE SNAKEROOT TOGETHER WITH SALT OR SODA TO SHEEP.

Ewe No.	*Initial weight Pounds*	*Placed on feed*	*Date of death*	*Days before death resulted*	*Weight at death Pounds*	*Feed consumed Grain Pounds*	*Feed consumed Weed Pounds*
21	121	Sept. 18	Sept. 29	11	......	8	5.25
28	96	Sept. 18	Oct. 7	19	73.5	15	10.25
37	118	Oct. 20	Oct. 20	18	78.5	10	6

Ewe 21 consumed 8 ounces of salt and ewe 37 ate 12 ounces during the periods of 11 and 18 days, respectively, in which they were on the experiment. Ewe 28 ate with her feed 30 ounces of baking soda. Salt and soda in these amounts given along with a ration of grain and white snakeroot are therefore without apparent antidotal effect.

The early treatments employed by physicians were drastic, since among them were blood-letting and strong purgatives. Graff (1841) advised the use of calomel, whereas Drake (1841) and Philips (1877) both advised against it. The latter used strychnine apparently with benefit. The allaying of abdominal pain by counter irritation over the stomach was a much used measure. Another favorite measure, which is at present much used, is the giving of liberal quantities of brandy and honey or brandy and milk (Dumm, 1880). Sodium bicarbonate is believed by Walsh (1909) to be efficacious. Medical treatises as recent as that of Hare (1907)* give no specific, but simply state that the treatment is purely symptomatic and consists of the judicious use of stimulants and sedatives as they may be needed.

General Summary

1. A disease of domestic animals commonly called trembles, because trembling is the most prominent symptom, has been the subject of investigation at this Station for two seasons. This disease is also called milksickness since man may contract the disease by the ingestion of milk products from cows affected with trembles. Attention in this investigation has been directed primarily to the etiology, transmission, symptomatology, and pathological anatomy of the disease.

2. A study of the literature on this disease reveals the fact that it has existed in North Carolina since the days of the American Revolution, and that the first published account appeared in 1810. The disease was, no doubt, more commonly present during the early part of the past century than is now the case.

3. Many accounts have been published dealing with the causation of trembles and milksickness. Some of these causes are more or less fanciful, while

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*Hare, H. A. A text-book of the practice of medicine, 2d edit., 1907. Philadelphia and New York.

the others may be classified as supporting either (1) the mineral poison theory, (2) the germ or microbic theory, or (3) the poisonous plant theory.

4. The first claim that white snakeroot, Eupatorium urticæfolium, is responsible for the disease seems to have been made as early as 1840. The observations and experiments of several subsequent writers support this claim as do the present experimental studies.

5. There are about forty species of Eupatorium in the southern United States, none of which species except white snakeroot are known to be poisonous, although several species are used medicinally. White snakeroot is commonly present in shady, mountainous situations in western North Carolina, and is distinguishable with difficulty from some of the species of Eupatorium, except by one with experience in identifying plants and by the exercise of close observation.

6. In previous accounts, the symptoms of the disorder appear to have been described in detail only in the case of cattle and of man. The writers have not had occasion to see the disease in cattle or in man and in their studies have employed ewes, lambs, hogs, dogs, guinea pigs, and mice. Trembles was developed in sheep, hogs, and guinea pigs, and the symptoms of the disorder have accordingly been described with considerable fullness and illustrated photographically. The symptoms in sheep and hogs are sufficiently characteristic so that any one who has ever seen an affected animal may be certain of his diagnosis.

7. The disease may appear at any season of the year, but is most prevalent in late summer and autumn especially when other vegetation is scarce because of drought. The disease is frequently fatal in domestic animals while the sequel of milksickness in man, in case of recovery, is lasting debility.

8. The present experiments were conducted at West Raleigh, N. C., about 400 miles distant from the place of collection of the white snakeroot used, and no case of trembles or milksickness had ever appeared in West Raleigh prior to the performance of these feeding tests.

9. During the experimentation, 31 fatal cases of trembles and milksickness have been developed among the 44 ewes and lambs that were employed in some phase of the experimentation involving the feeding of white snakeroot. Two of these lambs contracted genuine cases of milksickness by suckling their mothers, demonstrating that the disease may be transmitted through the milk. This fact has for a long time been a matter of common belief among farmers. Furthermore, animals in lactation, having access to white snakeroot, may be apparently normal yet are capable of transmitting milksickness through their milk.

There was no other contributory cause of death of these 31 animals except in the case of two individuals, in which cases stomach worms may have been, in part, responsible for death.

One of the two hogs employed in the experiment developed a typical case of trembles.

Fifteen of the 29 guinea pigs died from the feeding of white snakeroot or its products.

It is not possible to determine with certainty whether death resulted from the feeding of butter in the case of two of the three mice employed, although it is logical, in view of the experiments with suckling lambs, to believe that the disease may be transmitted through the agency of butter.

No evidence has been secured that the flesh of sheep dead of trembles is capable of transmitting the disease to dogs, although 11 carcasses were eaten by 7 dogs.

Animals appear to differ greatly in their susceptibility to poisoning, since some became affected within a week after feeding on white snakeroot was begun, others only after several weeks, and a few remained unaffected.

10. No detailed studies of the pathological changes accompanying this disease, other than those of Jordan and Harris, have been made prior to the present study. The reports on previous studies seem all to be couched in vague, general terms. The present findings agree essentially with those of Jordan and Harris.

11. No febrile condition is present in affected sheep. The most prominent changes noted in the 22 autopsies on sheep which were performed consist of albuminoid and fatty metamorphoses of the parenchymatous organs. The lesions commonly include petechiation of the epicardium; the presence of a metallic color of the kidneys, which exhibit active and passive congestion and cloudy swelling; a "nutmeg" appearance of the liver which has undergone fatty changes, and is congested so that blood drips freely from the sectioned surface; the occurrence of muco-enteritis of the small intestine; and the presence of a congestive condition of the brain and meninges.

12. All attempts to isolate a specific organism from the internal organs of six sheep, one hog, and one guinea pig were unsuccessful. This is supported indirectly by failure of diseased animals to communicate trembles to healthy ones when they are confined together and fed from the same trough.

13. The active principle is probably glucosidal in nature.

14. The sodium compounds, salt and soda, appear not to possess antidotal effects. In view of the grave cellular changes within the internal organs, there is little hope of the efficacy of medication with domestic animals and preventive rather than curative measures are to be employed.

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EXPLANATION OF PLATES

PLATE 1.

Eupatorium urticæfolium, or white snakeroot, showing the character of the leaves and flowers.

PLATE 2.

FIG. A—Ewe 161 in a characteristic standing posture assumed when the animal is seized with a paroxysm of trembling.

FIG. B—The same animal a few minutes later when the tremors have become more acute. The animal here, as in Fig. A, has spread apart the feet in an effort to remain standing.

PLATE 3.

FIG. C—At this stage of trembling, a few seconds subsequently, the animal is beginning to drop down to a resting posture.

FIG. D—A characteristic resting posture assumed by Ewe 161 after a violent spasm of trembling. Note that the eyes are closed, the ears droop, and the chin is resting upon the ground.

PLATE 4.

FIG. E—Ewe 12 in another resting posture commonly assumed by affected animals. The same evidences of stupor are present, but the head and neck are extended.

FIG. F—A state of coma has set in and the animal (Ewe 12) is no longer able to rise.

PLATE 5.

FIG. G—Lamb 244 in a comatose condition, and her mother (Ewe 171) who is entirely normal.

FIG. H—Guinea pig affected with trembles. This animal is unable to control its movements, and general debility is indicated by the drooping ears and partially closed eyes.

PLATE 6.

FIG. I—A pig at the beginning of trembling.

FIG. J—The tremors have become so violent within a few moments that the animal has settled backward upon her haunches and is squealing.

PLATE 7.

FIG. K—A resting posture assumed by falling forward from the position in Fig. J.

FIG. L—The same animal, prostrate. This position may be taken following Fig. K, or the animal may topple over from a standing posture.

PLATE 8.

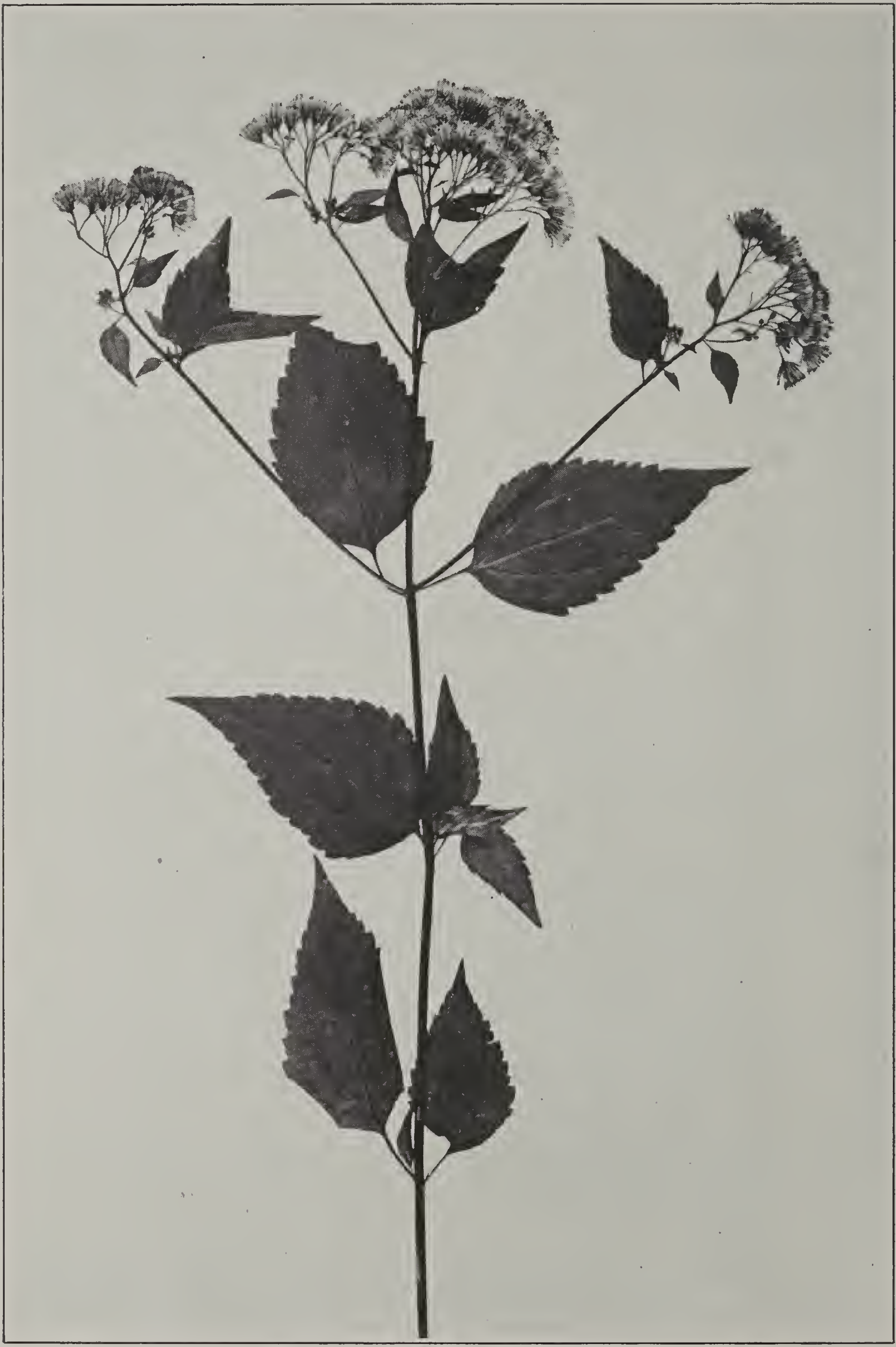
FIG. M—Acute parenchymatous nephritis. From Lamb No. 244. *a*, tubule in earlier stage of cloudy swelling; *b*, a tubule in advanced stage of cloudy swelling; *c*, an area infiltrated with round cells and polymorphonuclear leucocytes.

PLATE 9.

FIG. N—Glomerulitis and cloudy swelling of Lamb No. 200. *a*, glomerule; *b*, tubules in state of cloudy swelling.

PLATE 10.

FIG. O—Tube casts from urine of Lamb No. 200 (*a*).



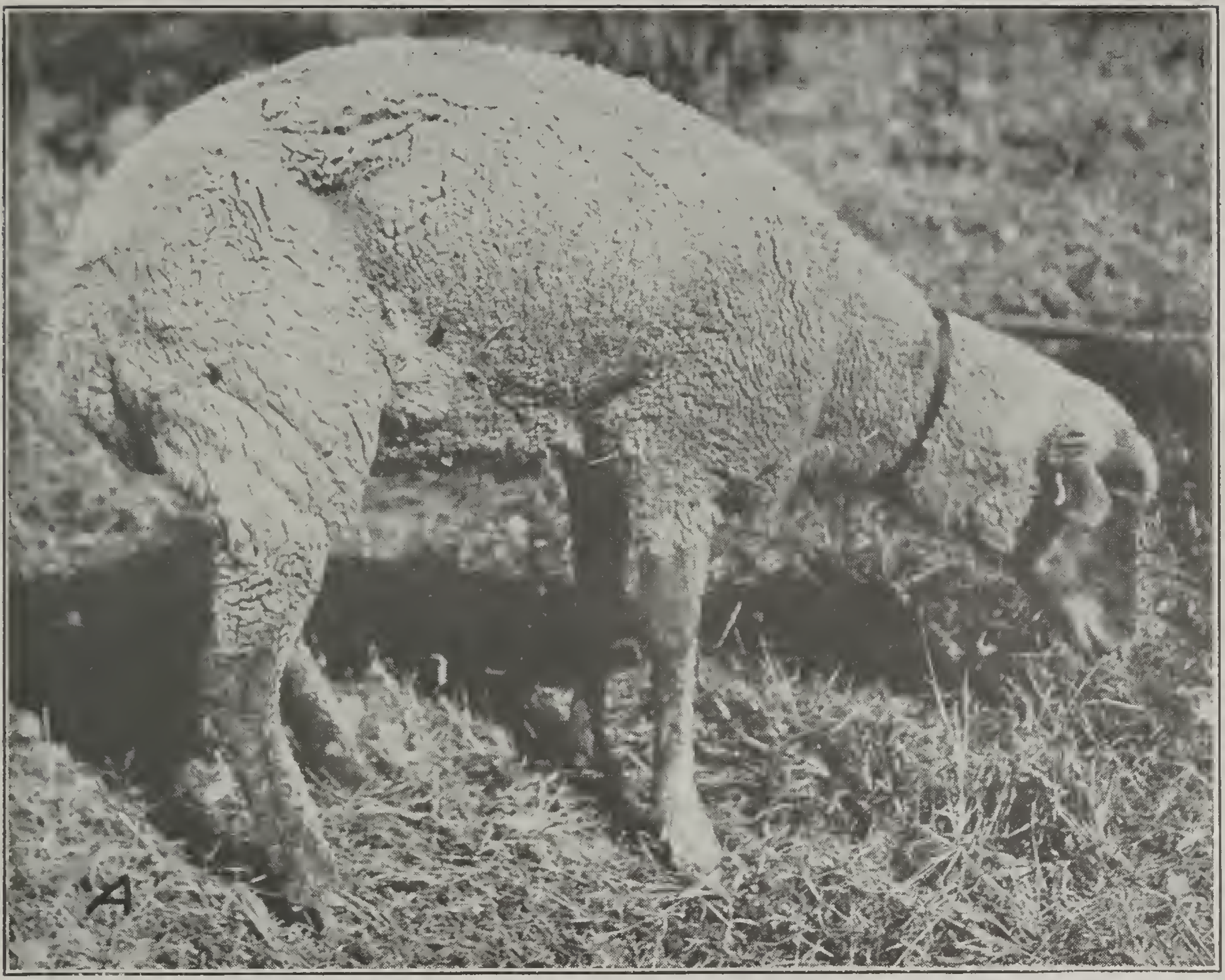


FIG. A

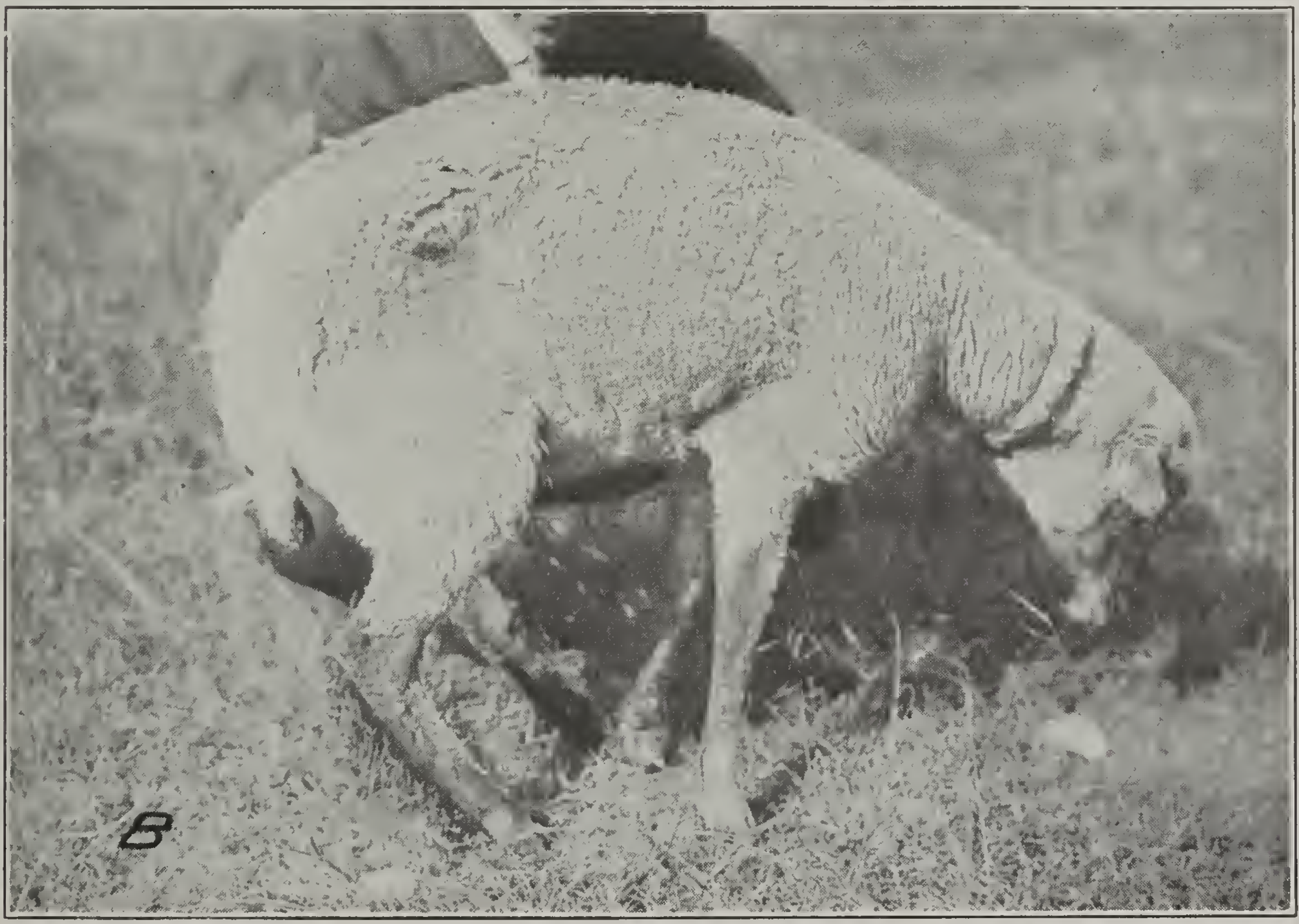


FIG. B

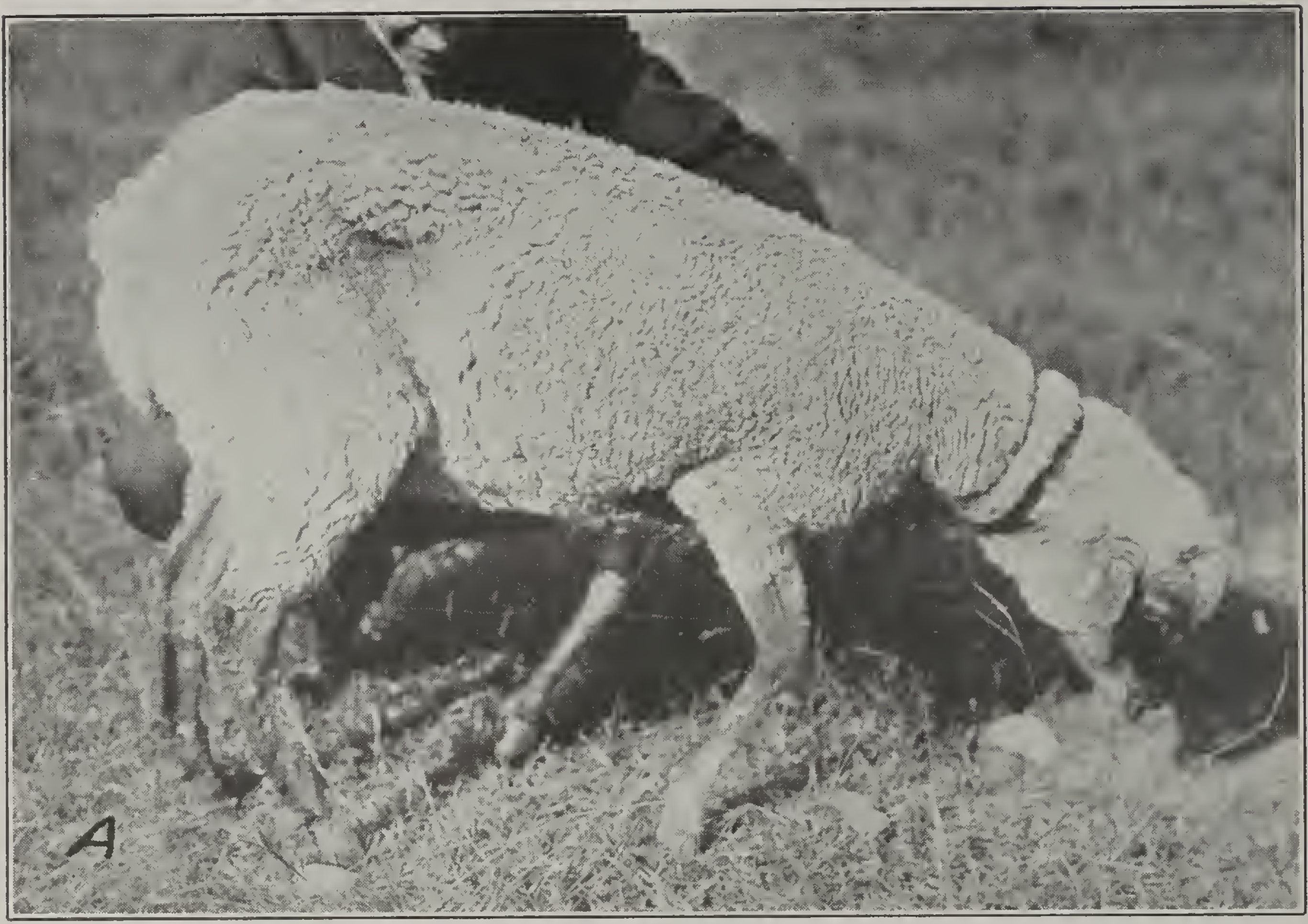


FIG. C

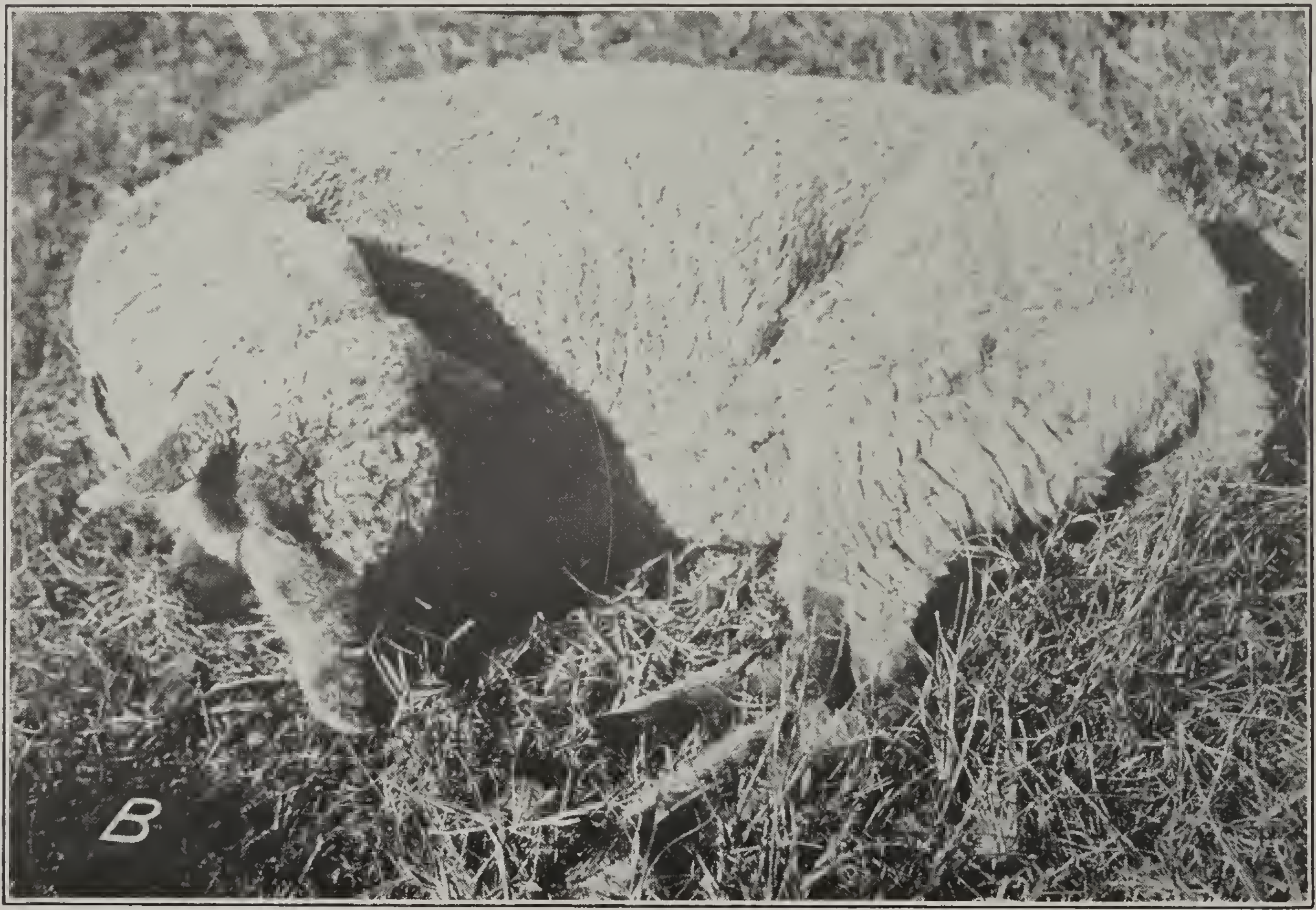


FIG. D

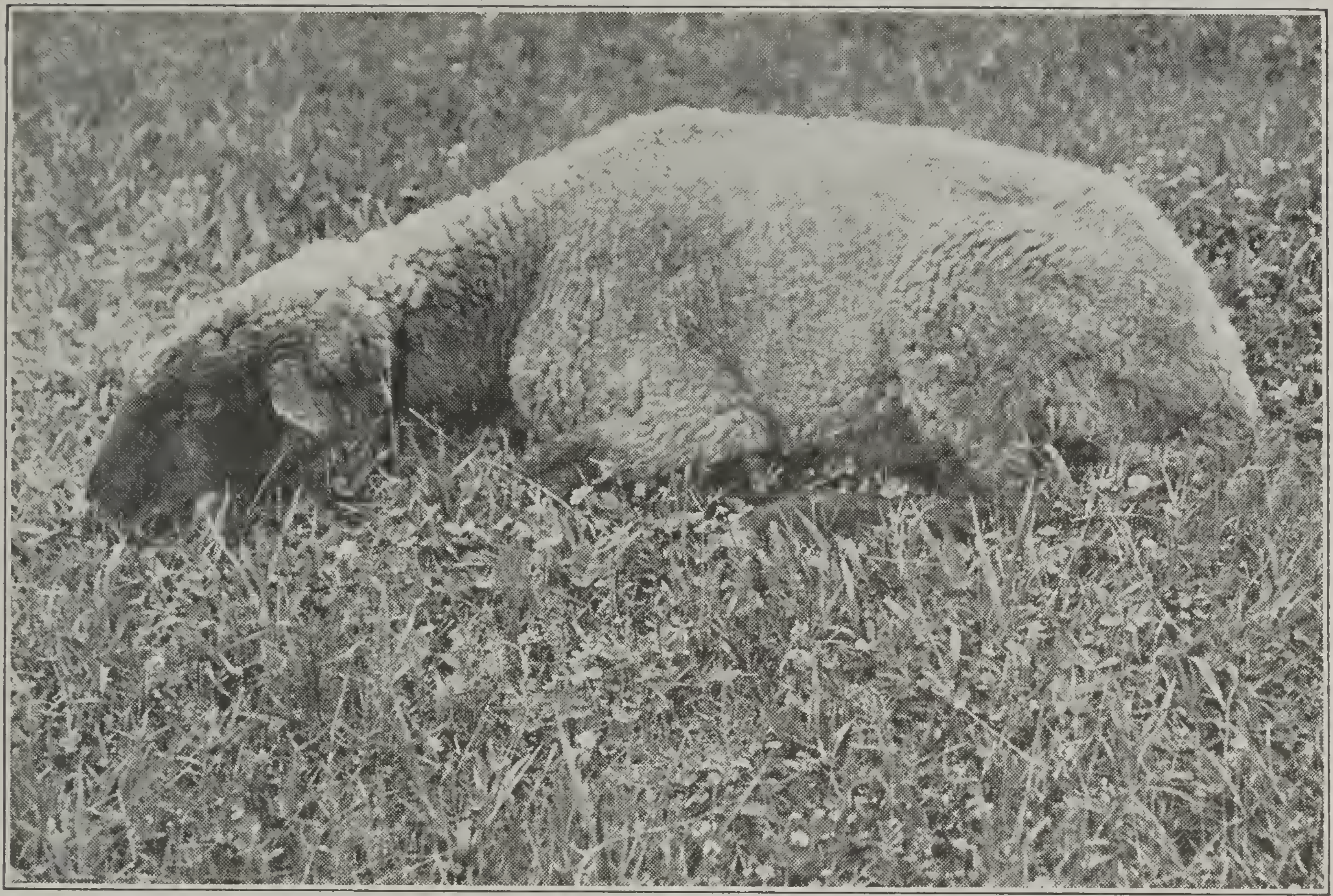


Fig. E

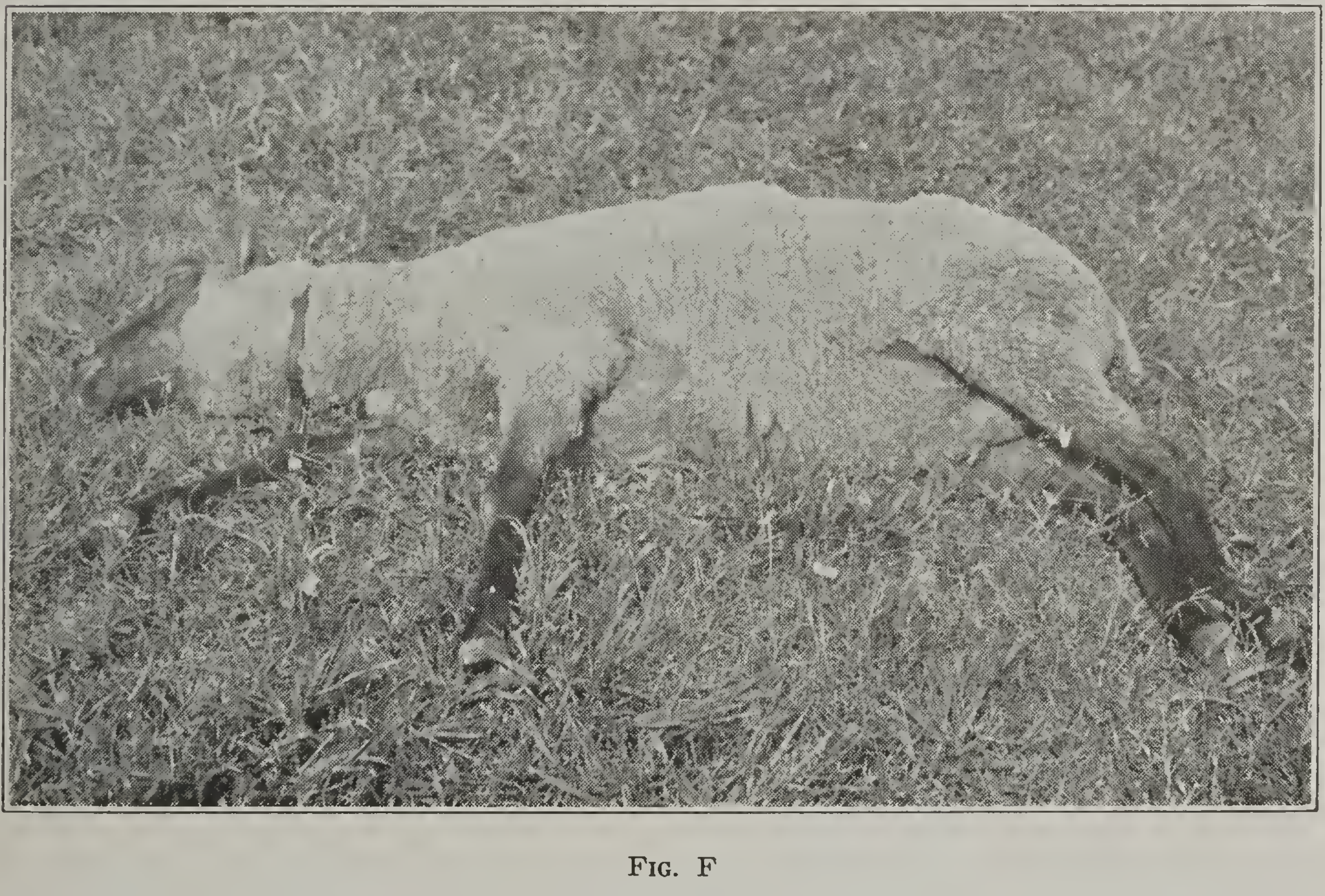


Fig. F

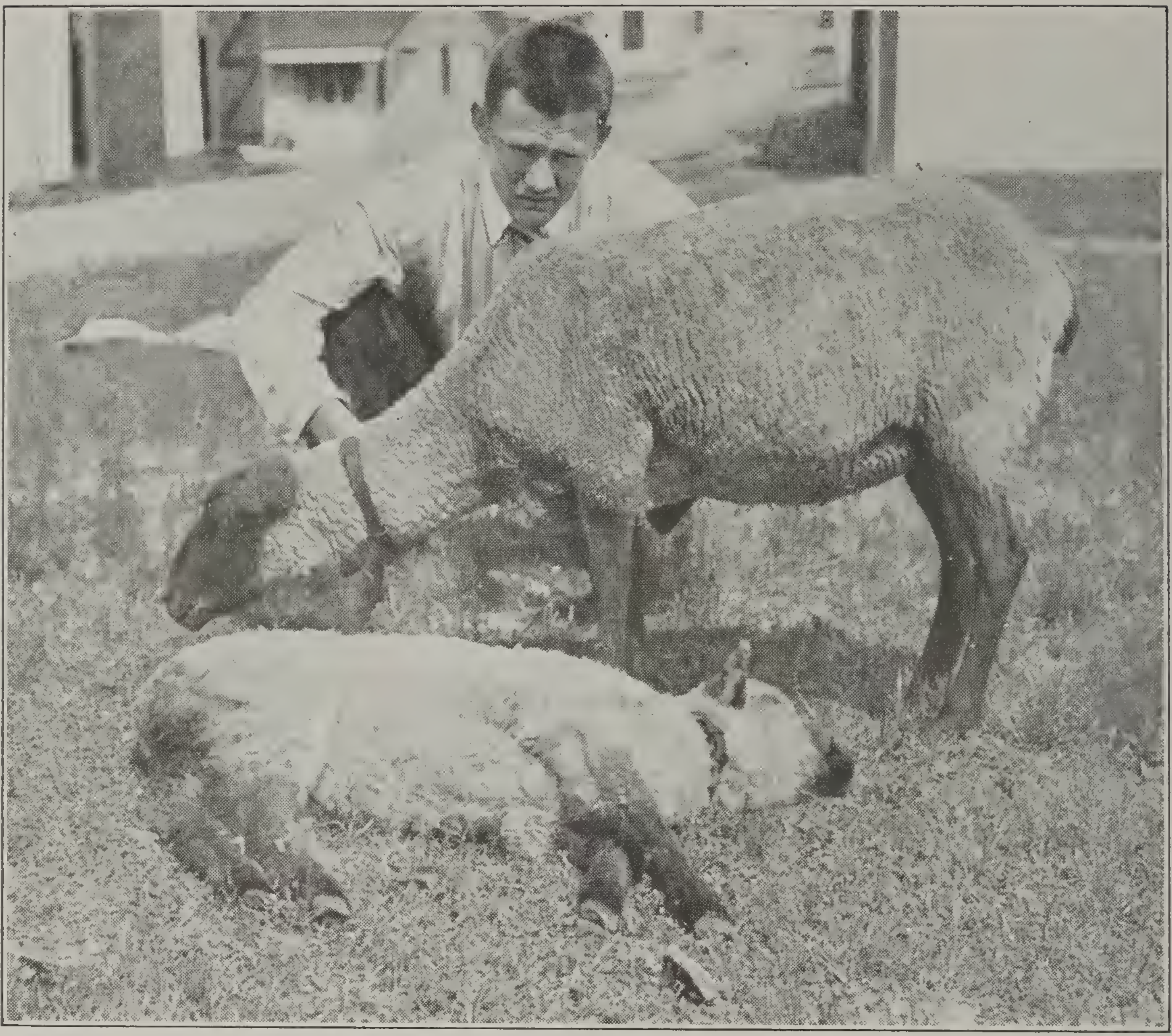


Fig. G

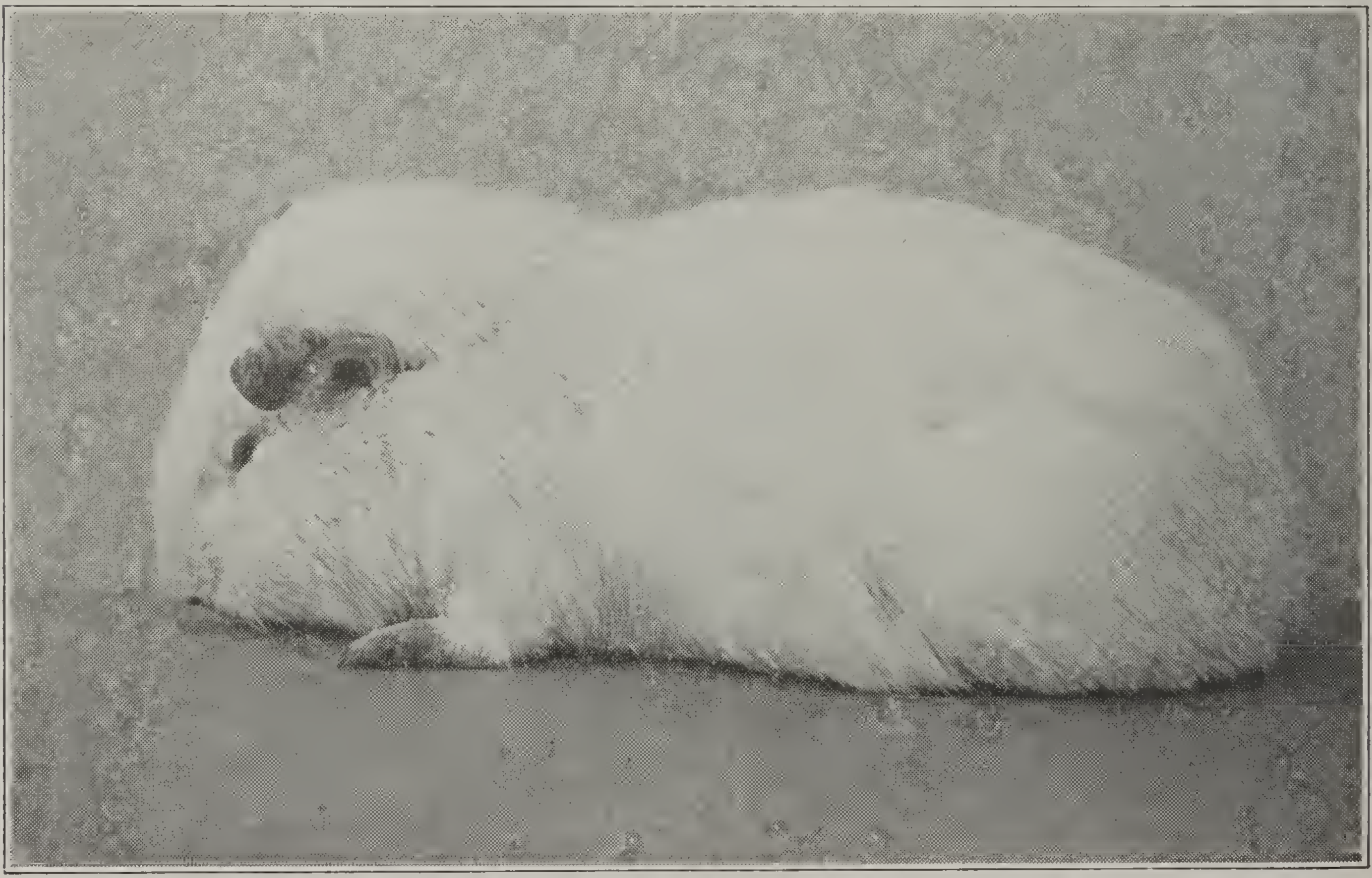


Fig. H

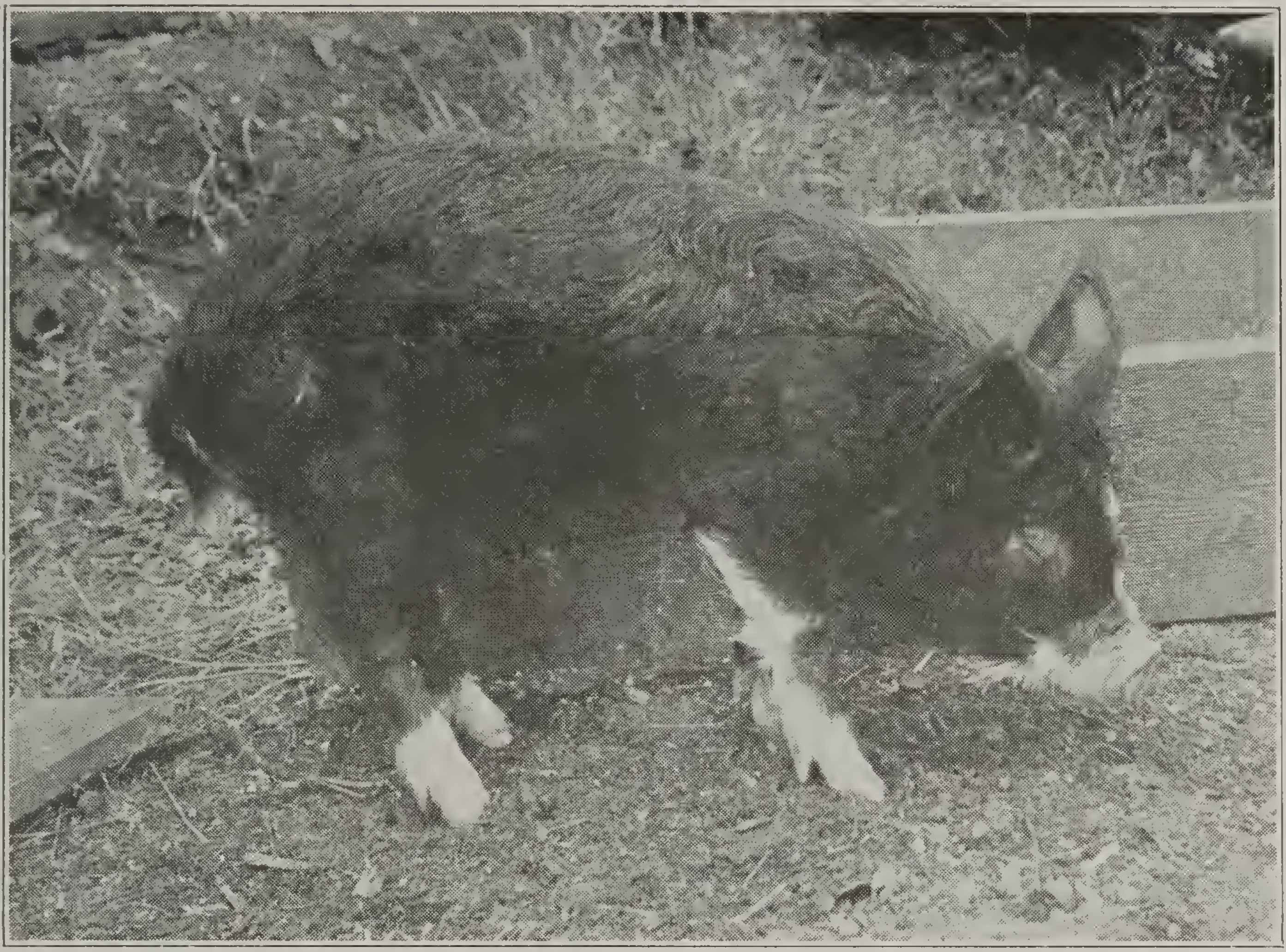


FIG. I

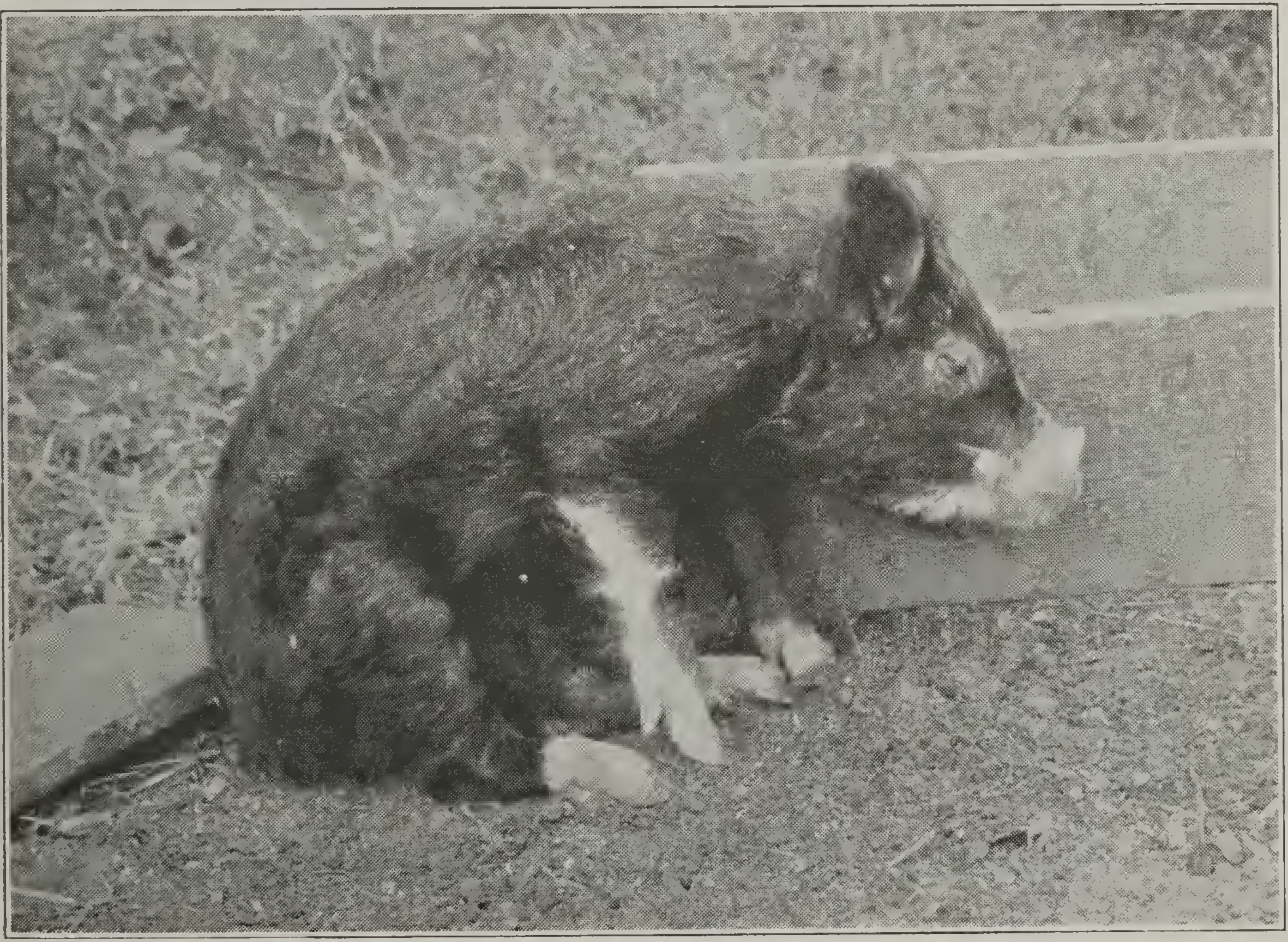


FIG. J

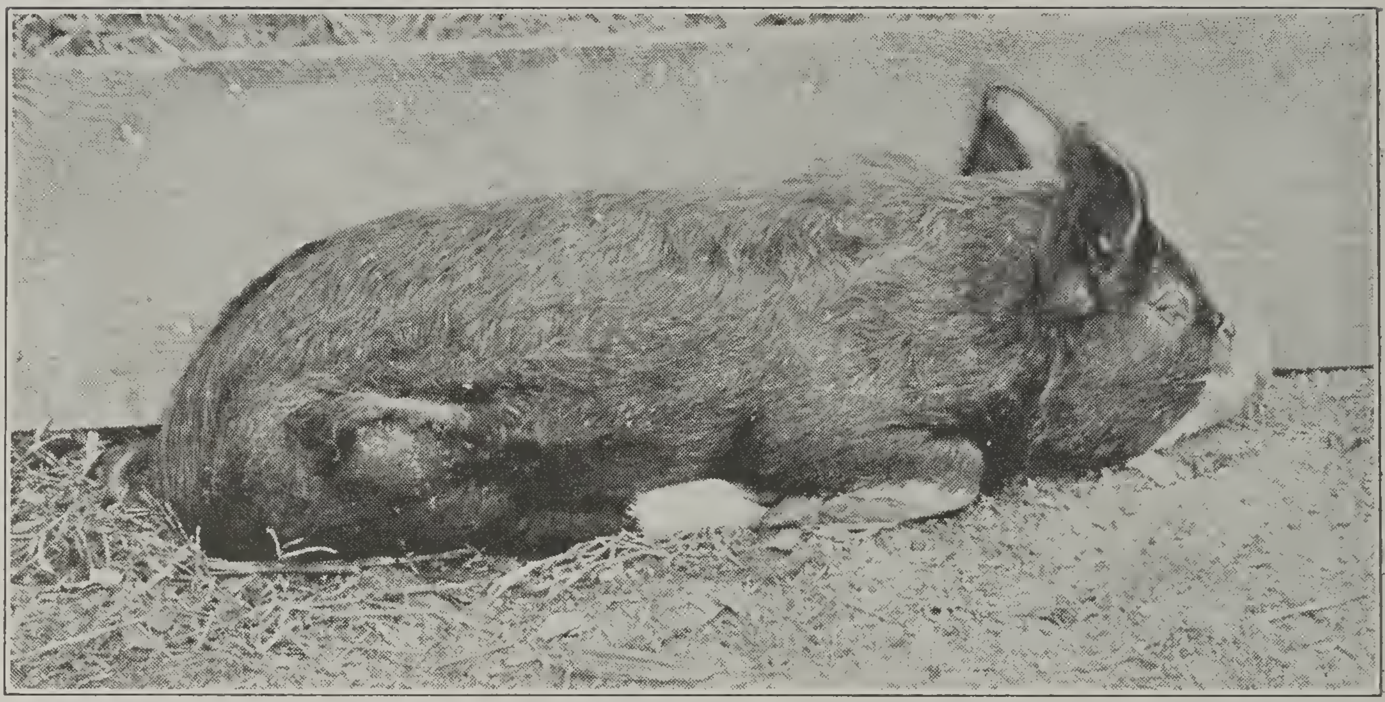


FIG. K

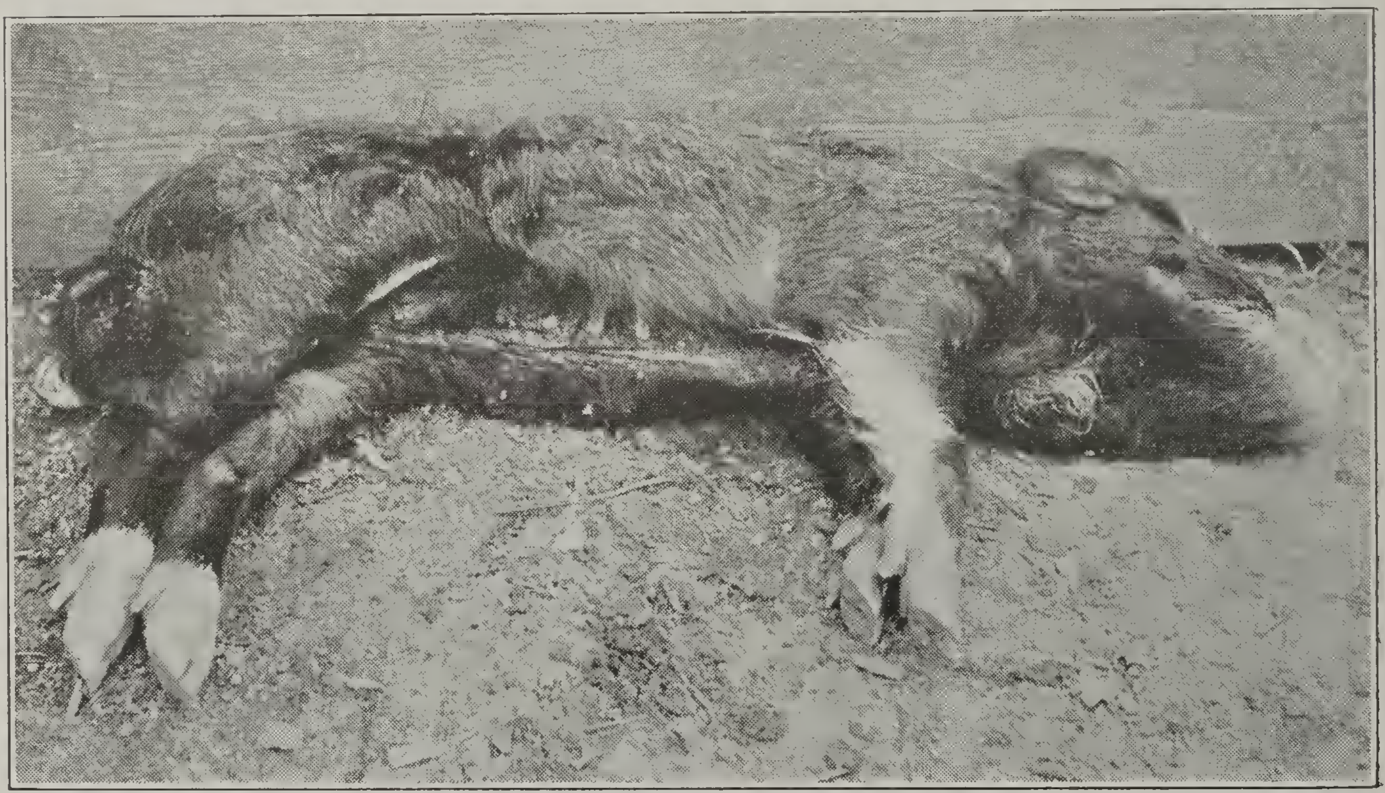


FIG. L

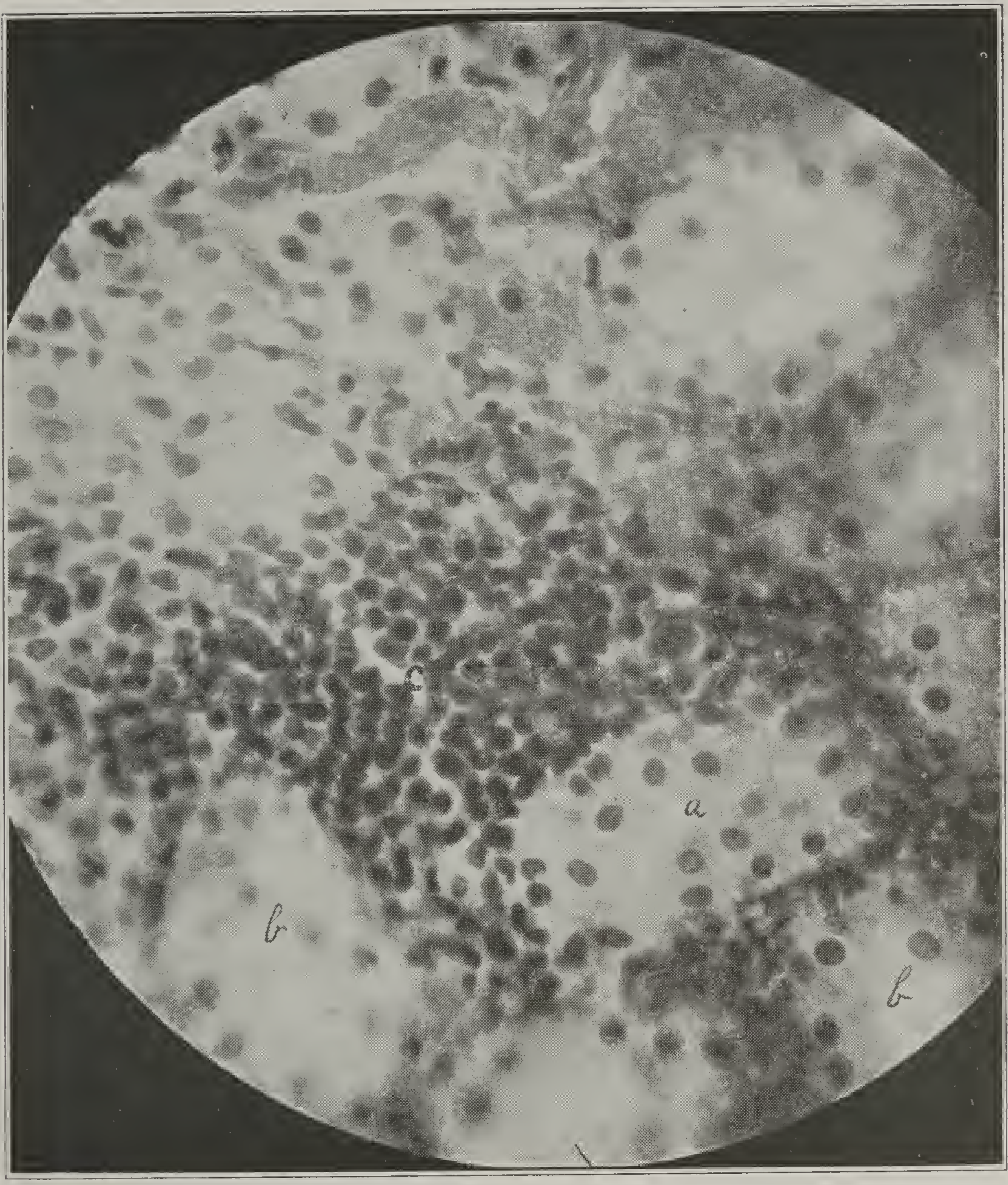


FIG. M

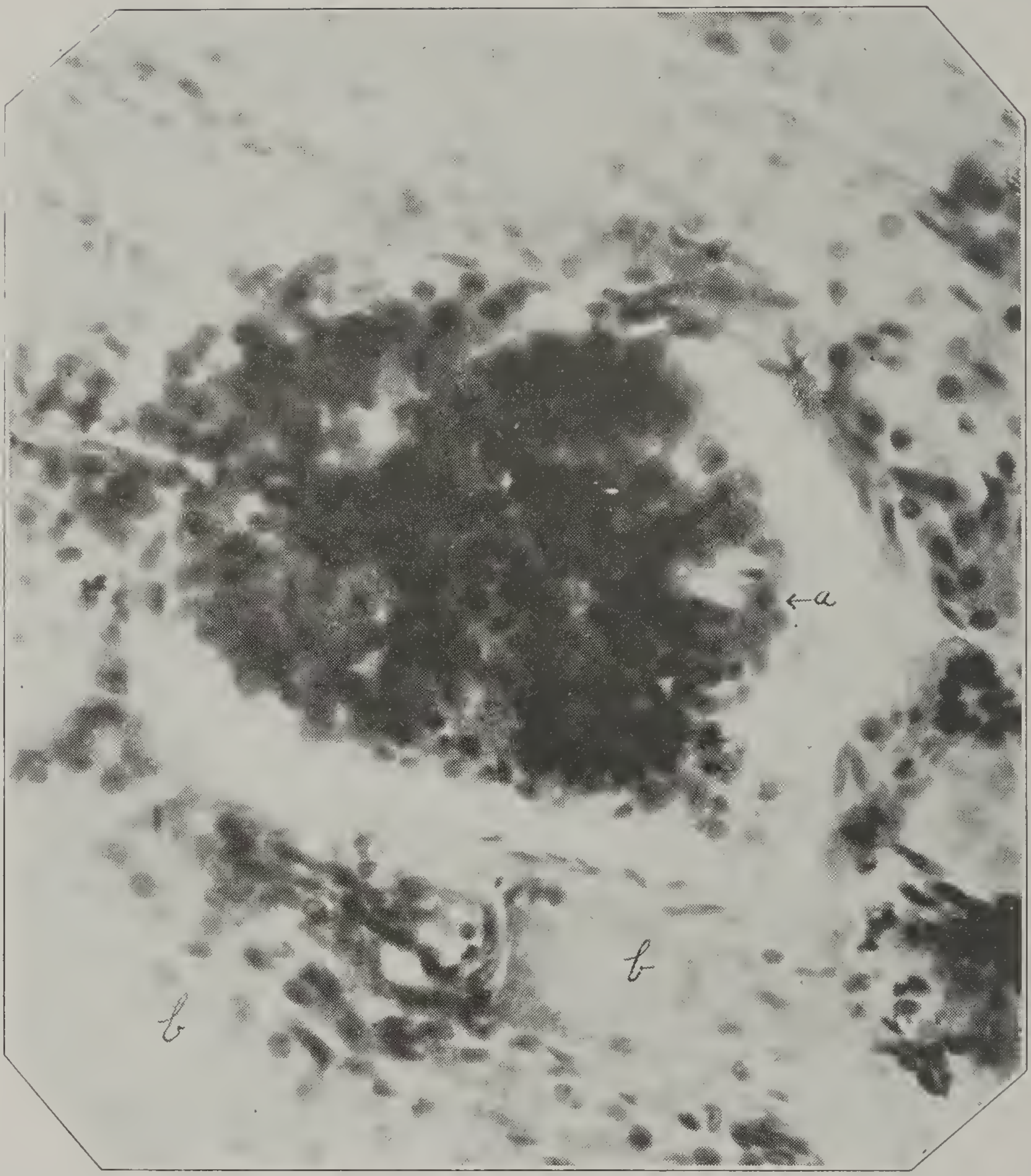


FIG. N

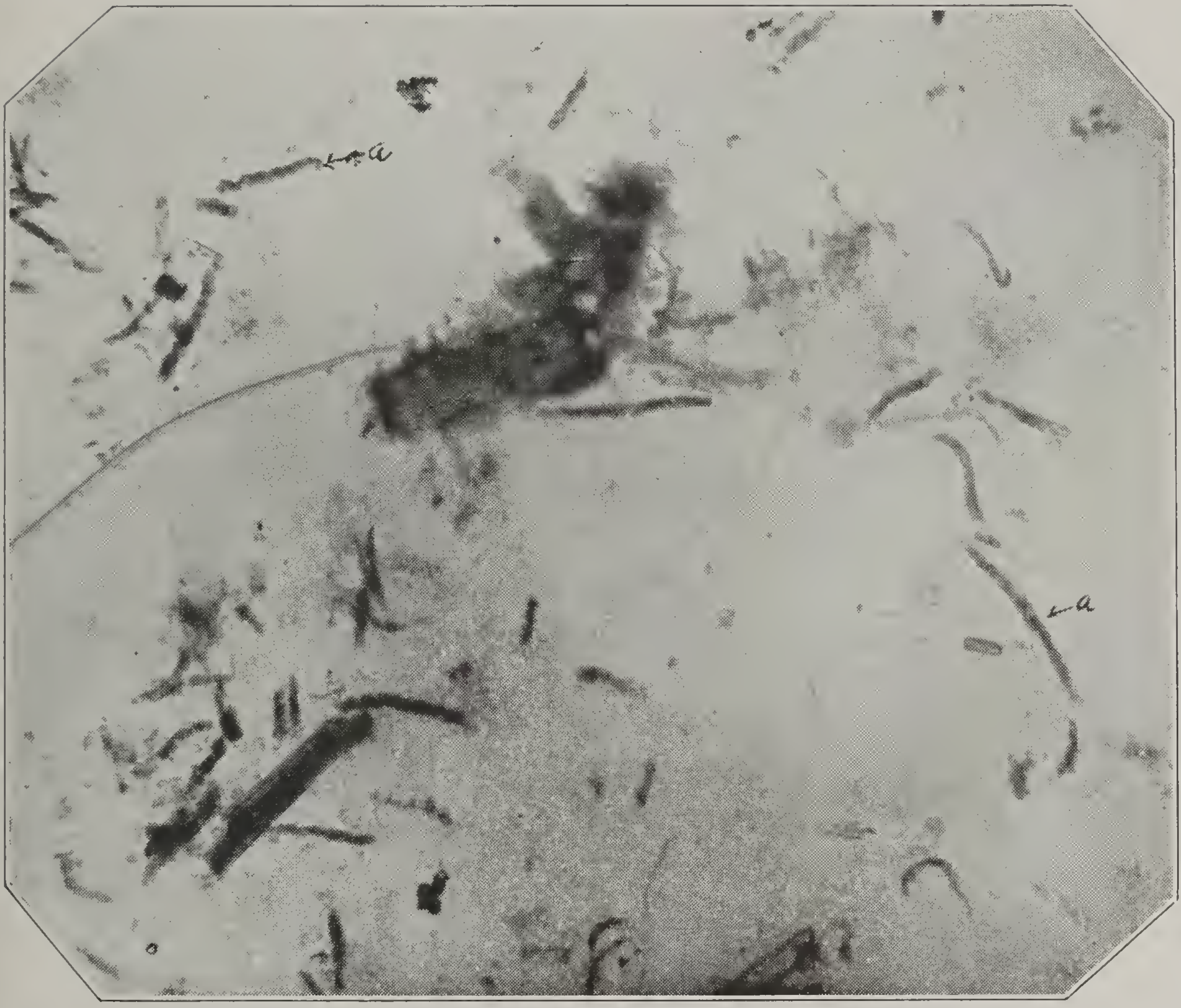


FIG. O

JUNE 1919 TECHNICAL BULLETIN No. 16

CLOVER STEM ROT

F. A. WOLF AND R. O. CROMWELL
Plant Pathology Division

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
Conducted Jointly by the
STATE DEPARTMENT OF AGRICULTURE
and the
NORTH CAROLINA STATE COLLEGE OF
AGRICULTURE AND ENGINEERING

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CLOVER STEM ROT[1]

By

FREDERICK A. WOLF AND R. O. CROMWELL

INTRODUCTION[2]

There has been present for several years in North Carolina a stem rot disease of crimson clover, Trifolium incarnatum. This disease has attracted attention because it results in the death of all plants in well-defined areas, involving in some instances, spots of considerable extent. Numerous inquiries as to the cause of this condition have been received during the past few years at the North Carolina Experiment Station and have led to an investigation of the nature of this stem rot disease. Furthermore, the growing importance of crimson clover as a cover crop and as an invaluable means of increasing and maintaining soil fertility makes it desirable to circulate the facts established by this investigation as widely as possible. It seems advisable, therefore, to present at this time, data bearing upon the history and distribution of the disease, on the relationship and life history of the causal organism, and on methods for the prevention and control of its ravages.

Historical

The disease in epidemic form has long been known in Europe and was observed as early as 1857 (14, 15, and 18) near Beberbeck, in the province of Hesse, Germany. Here it appeared in a field seeded with a mixture of red clover, Trifolium pratense and white clover, Trifolium repens, and was noted to be more destructive during the moist winter weather than during the preceding dry autumn. In 1870 it was first noted in Denmark and by 1878 it had spread to Sweden.

Two extensive accounts of this disease in Europe have appeared, one by Rehm (18) in 1872 and the other by Eriksson (6) in 1880. The first of these deals primarily with the morphology and development of the causal organism which is identified as Peziza ciborioides Fr. It also contains suggestions for the control of the disease and a record of observations upon the host species which were found to include red clover, white clover, crimson clover, and alsike clover, Trifolium hybridum. Among the other leguminous plants which were exposed to infection, but which remained healthy were alfalfa, Medicago sativa, sainfoin, Medicago falcata, black medic, Medicago lupulina, Onobrychis sativa, Ornithopus sativa, white sweet clover, Melilotus alba, and blue sweet clover, Melilotus caerulea. The other account deals with the distribution of stem rot and with the life history and nomenclature of the fungus which is designated, Sclerotinia trifoliorum Erik. In 1890 (19) the disease was reported as more destructive to Medicago lupulina in Denmark than to species of Trifolium. Subsequent accounts by European investigators deal primarily with experimental procedures looking toward the control of the disease.

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[1] Submitted for publication December 10, 1917.

[2] The junior author is now at the Iowa Agricultural Experiment Station, Ames, Iowa.

Stem rot has not only been prevalent in continental Europe, for a long time, but it very probably was the cause of lands becoming "sick of red clover," hence the name clover-sickness in the British Isles, as early as the early part of the nineteenth century. Investigations on clover sickness were begun at the Rothamsted Experiment Station, England, in 1849 (12) but the relation of the disease to the fungus Sclerotinia trifoliorum was not established until 1897 (3). English writers had previously expressed a variety of views in regard to the cause of clover sickness. Carruthers (3) at this time ascribed the disease to the fungus in question and suggested methods for its control.

The first observation of the occurrence of clover stem rot within the United States appears to have been made in 1890 in Delaware (2). While the disease was during subsequent years noted in several other States, it appears not to have been sufficiently destructive to incite investigation. In 1914-1915, however, it was reported by Gilbert and Myer (10) to be productive of serious damage to young alfalfa plants in Kentucky. This report and a subsequent one (11) constitute the only American accounts which indicate any considerable study of the disease.

Distribution of the Disease

As indicated above, the disease is known to occur abroad in Germany, Denmark, Sweden, and England, and in America within Delaware and Kentucky. It has furthermore been recorded from Canada, New York, New Jersey, Virginia, Indiana, Oregon, and North and South Carolina. Its prevalence in these states indicates that it very probably occurs in other of the states where clover is grown. Its distribution within North Carolina is not completely known, but since clover is generally grown throughout the State and since the points from which collections of diseased material have been made are quite widely separated, it is believed that the disease is generally prevalent. As indicated by these collections the disease is known to occur within thirteen counties, namely: Alamance, Chatham, Columbus, Edgecombe, Forsyth, Granville, Halifax, Lincoln, Mecklenburg, Moore, Orange, Rowan, and Wake.

Host Plants

The disease has been shown by European writers to attack red clover, white clover, crimson clover, alsike clover, sainfoin and alfalfa. To this list Gilbert and Myer (10) have added spotted spurge, Euphorbia maculata. Frank (7) observed a sclerotial disease of peanuts of whose identity he could not be certain since he did not have the apothecial stage, but he may have been dealing with the same organism.

Names of the Disease

As is the case with many plant diseases, various common names have been applied to this disease, although the name stem rot seems to be most appropriate. Among other names which have been employed are clover rot, wilt, and root rot. In England the name clover sickness has long been employed and other European accounts make use of the names Sclerotium rot, clover rot (Kleefaule), and clover canker (Kleekrebs).

Description of Clover Stem Rot

Stem rot on crimson clover can first be noticed in the fall when the plants are still small. It continues to spread, however, when weather conditions permit, throughout the winter months and is destructive until the time when unaffected plants are mature. The first evidence of the presence of the disease is manifested by a wilting of the stem and leaves. These portions then turn yellowish, rather quickly succumb and become brown (Plate 3, Fig. 1). Closer examination discloses the fact that the stems near the base of the stool or at the ground level are involved in decay, which begins as a slight discoloration and proceeds until the stems have rotted off. At this time a dense, white, mold-like growth is prominently present at the base of affected stems. In the presence of a suitable supply of moisture this fungous growth becomes more profuse and within a few days, compact masses of hyphae will have formed on the surface of the decaying stems (Plate 3, Fig. 1). These masses soon become black in color and of a cartilaginous consistency and are the sclerotial stage of the causal organism.

These black sclerotia are sometimes as large as a pea and their presence can be used as an aid in a field diagnosis of the disease. With the maturing of these sclerotia and the simultaneous disintegration of the stems, the sclerotia remain scattered over the soil where the plant has been. Meanwhile, the roots also are involved in decay and the sclerotia which are formed as the tissues become decomposed, remain in the soil. Since the disease spreads outward from a center of infection, plants in all stages of the disease may be found in any one spot. When the diseased areas are large the centers may be entirely bare or there may remain the debris of the badly decayed stems. The stand of clover, in case plants die in localized areas, is thus rendered not uniform or when the centers of infection are numerous and extremely favorable conditions prevail, the stand may be rather uniformly destroyed.

Etiology

Clover stem rot is caused by a discomycetous fungus, Sclerotinia trifoliorum Erik. This organism is identical with the one which was first illustrated by Hoffmann (13) in 1863, but to which he applied the name Peziza ciborioides Fries. Furthermore, it is the same as the one which is so fully described by Rehm (18) and which he designated as Peziza ciborioides Fries. The organism which was originally identified as Peziza ciborioides by Fries (9) in his Systema Mycologicum, as has been pointed out by Eriksson (6), is entirely different from the clover stem rot fungus. In the first place, Fries makes no mention in his description of Peziza ciborioides of sclerotia, structures which could not have escaped his notice had he been working with the fungus on clover. Then, too, he states that the fungus grows early in spring upon stems in moist places and heathes, whereas the clover rot fungus appears in Sweden during the fall upon the collar of clover plants. Both the time of appearance and the habitat of the fungi, therefore, fail to accord. It was for these reasons that Eriksson regarded Peziza ciborioides Fries as applying to a form entirely distinct from the clover stem rot organism.

Güssow (12) has suggested that the combination Sclerotinia ciborioides Rehm is the most tenable name. In the light of the fact, however, that Rehm (18) misidentified Fries' species there appears to be little reason for retaining the specific name ciborioides, when the proper generic name Sclerotinia instead of Peziza is employed.

Morphology of Sclerotinia Trifoliorum

Although several careful investigations on the morphology of the stem rot fungus have been reported, this phase of the problem is given attention at this time because of certain statements in recent bulletins and text-books bearing on the relationship of Sclerotinia trifoliorum and Sclerotinia libertiana. In a bulletin dealing with wilt of alfalfa in New York, for example, Stewart, French and Wilson (22) state "that the fungus causing the disease is supposed to be the Sclerotinia trifoliorum Erik, given in all text-books on plant diseases as the cause of a stem rot of clover and which is said to attack also alfalfa. However, Prof. R. E. Smith, to whom specimens were sent, reported (20) that "the sclerotia were entirely similar to those of Sclerotinia libertiana and that they produce a Peziza form which leaves no doubt that the fungus really is Sclerotinia libertiana." Duggar (5) states that some regard the clover stem rot fungus as identical with Sclerotinia libertiana. Stevens (23) states that they are "by some regarded as identical; sufficient evidence has, however, not been adduced to prove them the same." More recently Smith (21), in an investigation of cottony rot of lemons in California, caused by Sclerotinia libertiana, has noted that the same organism attacks alfalfa and vetch. It seems desirable, therefore, that a comparative study of Sclerotinia trifoliorum and S. libertiana be made to establish their relationship to each other. They were accordingly isolated from crimson clover and from lettuce respectively and maintained in culture under observation for a considerable period.

The mycelium or vegetative body of the clover stem rot fungus appears under no conditions, except in the formation of sclerotia, to produce an abundant aerial growth on the surface of stems. It is then not loose and cottony, as in the case of lettuce drop fungus, in which there is a profuse mycelium, especially under conditions of high relative humidity. This is in accord with observations by Gilbert and Meyer (10) on Sclerotinia trifoliorum and Stevens and Hall (24) on Sclerotinia libertiana. Furthermore, constant differences in mycelial development are apparent in culture. On steamed cornmeal or potato agar, media noted to favor rapid growth of both organisms, S. libertiana makes the more luxuriant, rapid growth, a difference which persists even after sclerotial formation has been completed. Even though there is considerable variation in diameter of the hyphae, with an overlapping of extremes, the lettuce drop fungus possesses the larger, coarser mycelial threads. Comparative measurements of the younger growing tips, as shown in Plate 1, Fig. 1, indicate that the relative diameters are approximately in the proportion of 2 to 3.

It does not appear to be possible to differentiate between the peculiar processes termed attachments, which have been described in previous accounts and which develop when contact between hyphal tips and wall of the culture flasks or Petri dishes is effected. In the case of each organism, the hyphae become flattened, septate, profusely branched and interlaced resulting in the formation of dark masses which adhere to the glass surfaces.

The sclerotia of both are variable in shape, in that they are spherical, cylindrical, flattened or irregular, and in size which ranges in the case of S. trifoliorum from .3 to 10 mm. and to as large as 20 mm. in S. libertiana. In general, the latter is considerably larger as indicated in Plate 2, Fig. 2. No differences in development or structure were noted, however. From 2 to 3 weeks are required for their formation. They begin as dense, floccose, white mycelial masses. After a few days the masses have increased in size, become compact and of a cartilaginous consistency. At this stage they are white to pale cream-colored, which color persists within although the exterior rapidly changes to an inky black. In culture on flasks of steamed corn meal, very abundant sclerotial formation results, forming in some instances, a sclerotial crust over the entire surface in the case of the clover stem rot organism, whereas the sclerotia remain for the most part separated in S. libertiana.

No observable differences were noted in the size and manner of production of the apparently functionless microconidia. These structures are small, spherical bodies, 2 to 4 mikra in diameter, which are abstricted in acropetal succession from flask-shaped cells. These cells may appear as lateral or terminal branches from the vegetative hyphae, Plate 1, Fig. 2, or from the germ tubes produced in the germination of the ascospores. Microconidia may form in conspicuous, grayish, mealy patches on the surface of the mycelium in culture. They have not been observed to germinate except by the formation of a hypha 2 or 3 mikra long, nor has it been possible to bring about infections with them. This accords with the results by others, among whom are Coleman (4). During April, 1916, twenty crimson clover plants were sprayed with a suspension of microconidia from S. trifoliorum, were then covered to conserve moisture but no sign of infection was subsequently noted.

The macroscopic characters of the apothecia cannot be employed in differentiating the two forms. The stipe and disc are yellowish brown in color. The stipes are variable in length, depending upon the depth below the surface of the soil to which the sclerotia are buried. In extreme cases their length varies from 3 to 30 mm. and their thickness from 1 to 2 mm. The discs are at first deep cup-shaped, but expand to a flat disc, 3 to 10 m.m. in diameter, the margin of which may become reflexed and fissured, Plate 1, Fig. 4. Apothecia of both species have been secured by the writers by burying sclerotia from 2 to 9 months in moist sand in flower pots. Sclerotia of S. trifoliorum which had been matured in culture in July, 1910, were planted during September and they had developed fruiting discs by December, Plate 3, Fig. 2. Comparative measurements of asci and ascospores from these apothecia, as indicated in text, Plate 1, Fig. 3, show that the asci and ascospores of S. trifoliorum are very manifestly larger than those of S. libertiana which facts accord with observations of other investigators. These data are assembled in the following tabulation:

SCLEROTINIA TRIFOLIORUM			SCLEROTINIA LIBERTIANA		
AUTHOR	SIZE OF ASCI	SIZE OF ASCOSPORES	AUTHOR	SIZE OF ASCI	SIZE OF ASCOSPORES
Rehm	160x10*	15-17x8-10*	Saccardo ..	130-135x 8-10*	9-13x4-6.6*
Eriksson	180x12*	14-18x8*			
Kuhn	160-180x14*	16-20x8-10*	Stevens & Hall	82-2(?)	8.7-11.6x5.8*
Present writers...	140-160 8-11*	12-14x8*	Present writers...	95-120x 8-9*	7-9x6*

*Mikra.

Life History of the Causal Organism

The life cycle of the clover stem rot fungus is relatively simple since it possesses only one functional type of spore. It lives for the greater part of the year as a saprophyte and can maintain itself in the soil upon decaying plant tissues from one year until the next. On the basis of difference in appearance and function, the fungus may be regarded as possessing three stages. Under normal conditions the first or vegetative stage is present during late fall, winter, and early spring, within the clover plants causing their death. Invasion of the tissues begins at the collar, near the ground level, and extends upward and downward from this point of attack. During the same period the fungus continues its vegetative growth as a saprophyte in plants which it has killed. As the tissues are disintegrated the fungus appears at the surface of the decaying parts and becomes massed together in compact black sclerotia, the second stage. These sclerotia then remain dormant on the surface of the soil or within the soil in the spots left bare by the destruction of the clover. Since these sclerotia require only two or three weeks to mature they are being formed practically throughout the entire growing period of the clover crop. These sclerotia, which contain a reserve of nutriment, remain dormant and serve to carry the fungus over the summer season, or until opportune conditions for their growth again prevail. During the fall and early winter these sclerotia germinate by the formation of slender, yellowish-brown stalks. The distal ends of these stalks expand into the disc-shaped fruit bodies, which with the stalks constitute the apothecia, the third stage. From one to a half dozen apothecia are developed from each sclerotium, depending upon the size of the sclerotium. The upper or inner surface of these discs is lined with a layer of closely aggregated, elongated sacs, or asci, interspersed with the paraphyses. Rehm (18) has estimated that there are approximately 5,000 sacs in one square millimeter of surface, each of which bears at maturity eight ascospores. These ascospores are forcibly discharged, often in such numbers as to appear like small clouds of dust. They may then be carried by the wind to a moist surface and there germinate at once by the formation of a hypha. This hypha becomes, as growth proceeds, the vegetative mycelium, thus completing the cycle of development.

Whether these ascospores directly infect clover is not known, aside from the experimental work of Rehm (18) and Coleman (4). The former suspended mature fruit bodies over healthy plants and within 6 to 8 days the mycelium was present within the leaf blades, the plants subsequently collapsed, and sclerotia were formed in due time. He did not determine the mode of entrance of the germ tubes, but regarded it as probable that they entered through the stomates. The latter reported that young clover plants only can be readily infected by ascospores, although under favorable conditions older plants were in a few instances attacked. His investigations furthermore led him to assert that infection does not take place through the stomates, but by penetration of the epidermal cell walls. The results of direct infection of lettuce by ascospores of Sclerotinia libertiana led Stevens and Hall (24) to conclude that it seldom, if ever, occurs, and that the fungus becomes parasitic only after it has maintained itself for a time, as a vigorous saprophyte. Because of the fact that infection begin on the stems at or near the ground level in the case of stem rot of clover, it is believed that the vegetative mycelium is responsible for the disease and that little disease results naturally by direct infection with ascospores.

Infection Experiments

Several series of artificial inoculation tests have been made during the past two years. These involved plants of crimson clover, red clover, and lettuce grown within the greenhouse in flats or in beds, and crimson clover, hairy vetch, Vicia villosa, and lettuce grown under field conditions. Inoculation in the greenhouse was accomplished by introducing the appropriate organism from cultures on steamed rice or steamed corn meal or from diseased plant parts into the soil near the collar of the plants. Wilting was apparent within 8 to 10 days in the case of lettuce, crimson clover, or red clover, when either Sclerotinia trifoliorum or S. libertiana were employed. Practically all of the inoculated plants succumbed, irrespective of whether the inoculum consisted of pure cultures or of decaying plant tissues.

During April, 1917, plants of crimson clover and vetch five to eight inches in height were inoculated in the field by parting the stems and placing at the center of the plant fragments of lettuce affected with drop, S. libertiana. The plants thus inoculated were then lightly covered for 36 hours with a newspaper weighted at the corners. Five days after inoculation many of the younger stems near the center of the rosette were dead and their tissues involved in decay. The copious superficial mycelial growth was like that of the lettuce drop organism· Within twelve days even the larger stems had begun to wilt. Stem rot had never been present in this field and only the inoculated plants were affected. Furthermore, lettuce plants in another field were inoculated on the same day by inserting diseased lettuce leaves into the heads. These plants were in an advanced stage of drop a week later.

During November, 1917, crimson clover in the field was inoculated by placing in the soil pure cultures on corn meal of S. trifoliorum and of S. libertiana. Four weeks later circular areas eight to ten inches in diameter in which all of the plants were dead, had formed around the place of inoculations with each of the organisms. Lettuce plants, furthermore, became affected when diseased clover plants were transplanted beside them. These reciprocal inoculations leave no doubt that both clover and lettuce may be attacked by either S. trifoliorum or S. libertiana. Smith's (21) observations on the cause of cottony rot of lemons are of interest in this connection since he states that their decay is due to S. libertiana, although S. trifoliorum is able to develop on artificially inoculated lemons and may cause some of the infection. He also points out that alfalfa and vetch when grown as cover crops in lemon groves are attacked by S. libertiana. The confusion which has existed in the literature dealing with the identity and relationship of S. trifoliorum and S. libertiana is, therefore, clarified by these reciprocal inoculations which are in part a confirmation of studies and observations by Smith (21).

Means of Spread of Stem Rot

It seems highly probable that stem rot of clover was introduced into America from Europe, but the method of its introduction, together with the means for its spread into new localities each year, have remained more or less problematical. Eriksson (6) states that the fungus undoubtedly overwinters as hyphae adhering to the seeds and not as sclerotia or spores and that the disease is spread by this means.

Coleman (4) suggests that sclerotia mixed with the seed are a probable means of the distribution, although spores adhering to the seeds are also a possible means. Observations on this point, first made during the fall of 1914, by Prof. H. R. Fulton, then Plant Pathologist for the North Carolina Experiment Station, show that sclerotia are present in commercial crimson clover seed. He states:* "I received from county agents some six or eight samples of seed. Two of these, I distinctly remember, showed the contamination with sclerotia." If seed were harvested from affected fields, sclerotia would almost certainly be mixed with seed (Plate 2, Fig. 3), and would remain with them even though the seed were very carefully cleaned. Seed contaminated with sclerotia are, therefore, believed to account for the introduction and presence of stem rot of clover.

Numerous possibilities might be suggested to account for the local distribution of the disease. The fungus is known to spread through the soil along the margin of the diseased areas. Further, the sclerotia which normally remain dormant for a time, even though favorable conditions for their germination may obtain and which may retain vitality for at least two and one-half years under laboratory conditions and one and one-half years in the soil (18) may aid in its spread. They may be distributed by the implements used in cultivation, by the use of soil in inoculating with the nodule forming organism, by rains, or by being harvested with the hay crop. If this hay is fed to stock the possibility exists of the return of these sclerotia to the fields through the manure.

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*From a letter dated October 25, 1917.

The violent discharge of ascospores from the mature apothecia and the carriage of these spores by the air currents may also be taken into account in the local spread for short distances of the stem rot fungus.

Methods of Control

A very considerable body of data and observations bearing on methods of control have been presented in published accounts of stem rot. Much of the experimental work is of value, however, only inasmuch as it indicates methods which cannot be employed with success. In the light of the facts which have been presented relative to the life history of the causal fungus, some of these experimental results are to be anticipated. This may be instanced by the work with fertilizers and manures at the Rothamsted Experiment Station (16) which was based on the assumption that "clover sickness" is due to unfavorable soil conditions. No appreciable differences in treated and untreated plots were noted, results in agreement with those of Rehm (18) and others who have performed less extensive tests. Among the different fertilizers which have been applied are guano, barnyard manure, compost, bone meal, woodashes, gypsum, and lime. In the Essex experiments (1), where ground limestone was employed, clover remained free from disease. Causes other than the use of lime are believed to have been operative in this case, since these results do not accord with those just presented nor with the more recent ones of Coleman (4).

Among the cultural practices which have been noted to be of benefit by Freckmann (8) and Gilbert and Myer (10) are deep plowing. This was suggested from experiments by Rehm (18) in which sclerotia were buried at different depths in garden loam. No apothecia were developed when sclerotia were covered as deep as eight cm., about 3 inches. Rehm further pointed out that buried sclerotia were subject to destruction by earthworms, wireworms, millipeds, and mites. During the season of 1917 the sclerotia which were covered with sand in the studies on apothecial development were, in several series, noted to be completely devoured by fungus gnats, Mycetophilidae. The burial of sclerotia particularly when the soil is kept moist favors their decay from natural causes. Complete decay of the sclerotia of Sclerotinia trifoliorum was observed by the writers to be accomplished within two months. Stevens and Hall (24) have reported that under natural conditions the number of sclerotia of S. libertiana is greatly reduced by decay.

Precaution should be exercised both in the saving of seed from affected crimson clover fields and the planting of such seed because of its contamination with sclerotia. To guard against the introduction of stem rot into new fields or new localites, seed should be sampled and sent to a seed testing laboratory in advance of purchase. The introduction of the disease through manure from stock fed on hay from affected fields is also to be guarded against.

It is known that not all host species are equally subject to attack by S. trifoliorum, but whether or not any one of these species possesses marked varietal differences in susceptibility is not known. No experimental work appears to have been done on selection for disease resistance and the only observation that has come to our attention that such differences exist is by Nilsson-Ehle (17) who records that Swedish red clover of the variety serotinum is more resistant than exogenous strains.

In fields badly infested no measures can be relied upon to be effective other than the adoption of a proper rotation system in which clovers are not grown for a period of three or four years. Definite data are wanting on the length of time which the stem rot organism can live saprophytically in the soil, but it is logical to assume that it would no longer be present or the infective material would at least be greatly reduced in amount within a lapse of three or four years. Cowpeas and soybeans which are not subject to attack by Sclerotinia trifoliorum may well be used as a leguminous crop in such a system of rotation and winter oats and rye can be employed as winter cover crops.

Summary

1. Stem rot of clover is a fungous disease quite widely prevalent upon crimson clover within North Carolina.
2. Other kinds of legumes as red clover, white clover, alsike clover and alfalfa are subject to the same disease.
3. Stem rot occurs in several states and has been known in America since 1890. It was present in England over one hundred years ago, where it caused failures of red clover and was popularly called clover sickness. The disease was first reported in continental Europe in 1857.
4. The disease is prevalent from October to March and may be recognized by (1) a sudden wilting and death of plants in spots; (2) a rotting off or decay of the stems near the surface of the ground; (3) the presence of black sclerotia on the decaying stems.
5. The causal organism was first described in 1863 as Peziza ciborioides Fries. Since this was a mistaken identification it was in 1880 given the name Sclerotinia trifoliorum Erik, which name has been employed in the present account.

6. The fungus may be said to possess in its life cycle three stages, a vegetative or mycelial stage, a sclerotial stage, and an apothecial or ascogenous stage.

7. A study has been made of the development of S. trifoliorum from diseased crimson clover in comparison with S. libertiana from lettuce affected with drop. The two differ in luxuriance of mycelial growth, size of hyphae, size of sclerotia, and size of asci and ascospores.

8. In the infection experiments S. trifoliorum has been successfully inoculated into lettuce and crimson clover and S. libertiana into lettuce, crimson clover, and vetch.

9. The comparative morphological studies indicate that S. trifoliorum and S. libertiana are distinct species. Furthermore, the reciprocal inoculations clarify existing accounts of the identity and relationship of the two organisms.

10. The feature of most economic importance in the life history of the stem rot fungus is that the sclerotia may be mixed with the seed at time of harvest. The planting of such contaminated seed then insures the spread of the disease to new localities. Other agencies as implements, soil, and hay from infected fields may serve as a means of spread.

11. The sclerotia which remain dormant in infested soils constitute the means of keeping the organism alive where the disease has once appeared. Burial by deep plowing prevents their germination and thus prevents the spread of stem rot. Many sclerotia decay from natural causes and many are destroyed by insects.

12. Introduction of the disease into fields where it is not yet present should be prevented by avoidance of contaminated seed, by the exercise of care when soil is used to inoculate new fields with the legume nodule forming bacteria, and by not returning manure to clover fields when hay from infested fields has been fed.

13. The adoption of a system of crop rotation is the only reliable means of control for infested fields. Cowpeas and soybeans can be used in this rotation system to increase and maintain soil fertility, and winter oats and rye can serve as cover crops.

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EXPLANATION OF ILLUSTRATIONS

PLATE 1, FIG. 1—(a) Hypha of Sclerotinia libertiana and (b) of S. trifoliorum from the margin of colonies on potato agar showing relative size of the two species.

PLATE 1, FIG. 2—Microconidia (a) of Sclerotinia libertiana and (b) of S. trifoliorum borne on hyphal branches in old cultures.

PLATE 1, FIG. 3—Asci and ascospores (a) of Sclerotinia trifoliorum and (b) of S. libertiana, drawn with the same magnification.

PLATE 1, FIG. 4—Apothecia of S. trifoliorum developed from sclerotia.

PLATE 2, FIG. 1—Normal plant of crimson clover of the same age as the diseased ones shown in Plate 3.

PLATE 2, FIG. 2—Sclerotia (a) of Sclerotinia trifoliorum and (b) of S. libertiana, natural size.

PLATE 2, FIG. 3—Crimson clover seed contaminated with small sclerotia, which can scarcely be distinguished from the seed.

PLATE 3, FIG. 1—Plants of crimson clover affected with stem rot, S. triofoliorum, showing several stages of the disease. Sclerotia have formed on the stems as indicated by the direction of the arrows.

PLATE 3, FIG. 2—Apothecia of S. trifoliorum developed from sclerotia grown in pure culture and buried in sand.

PLATE 1

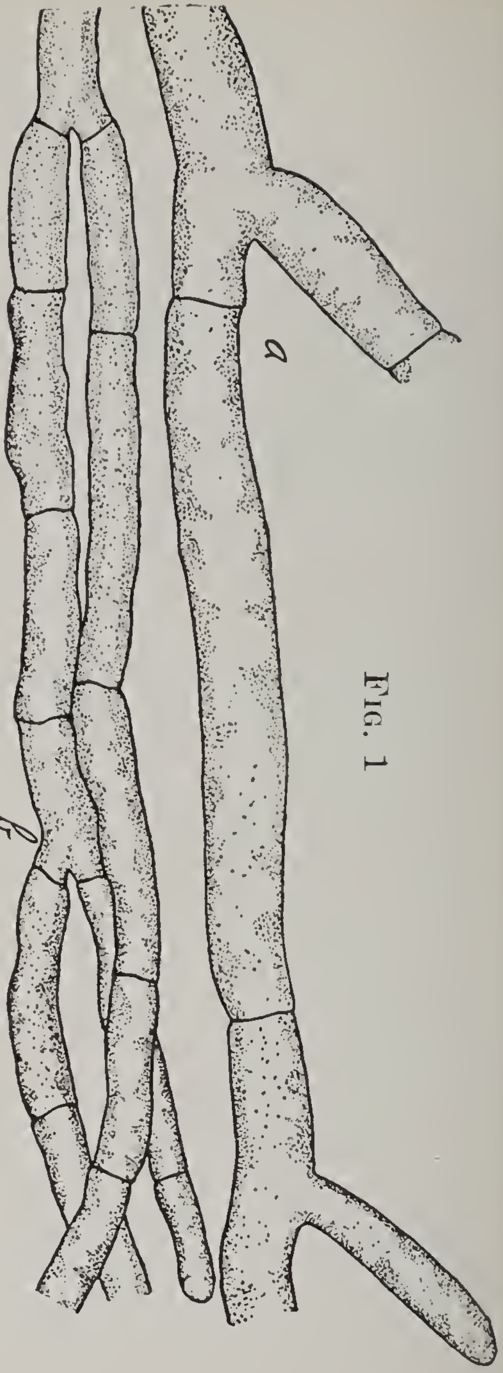

Fig. 1

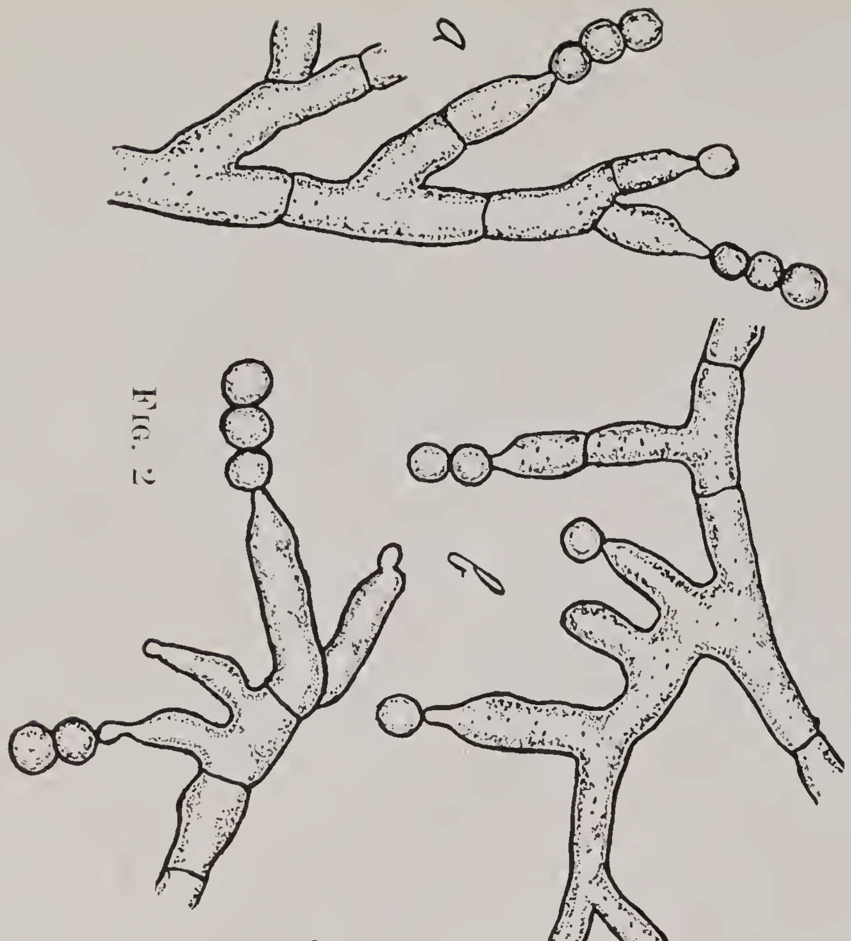

Fig. 2

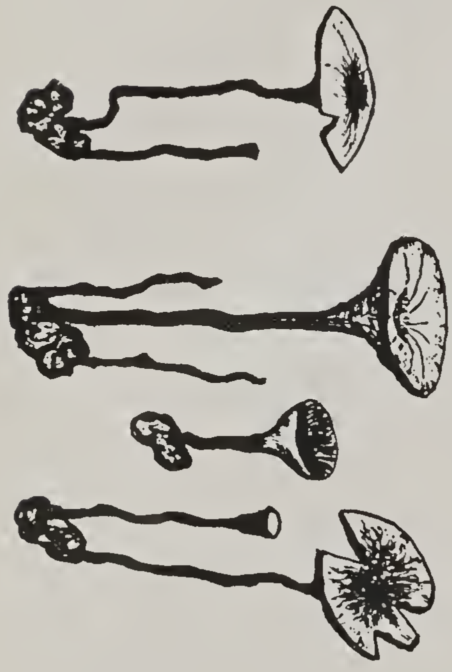

Fig. 4

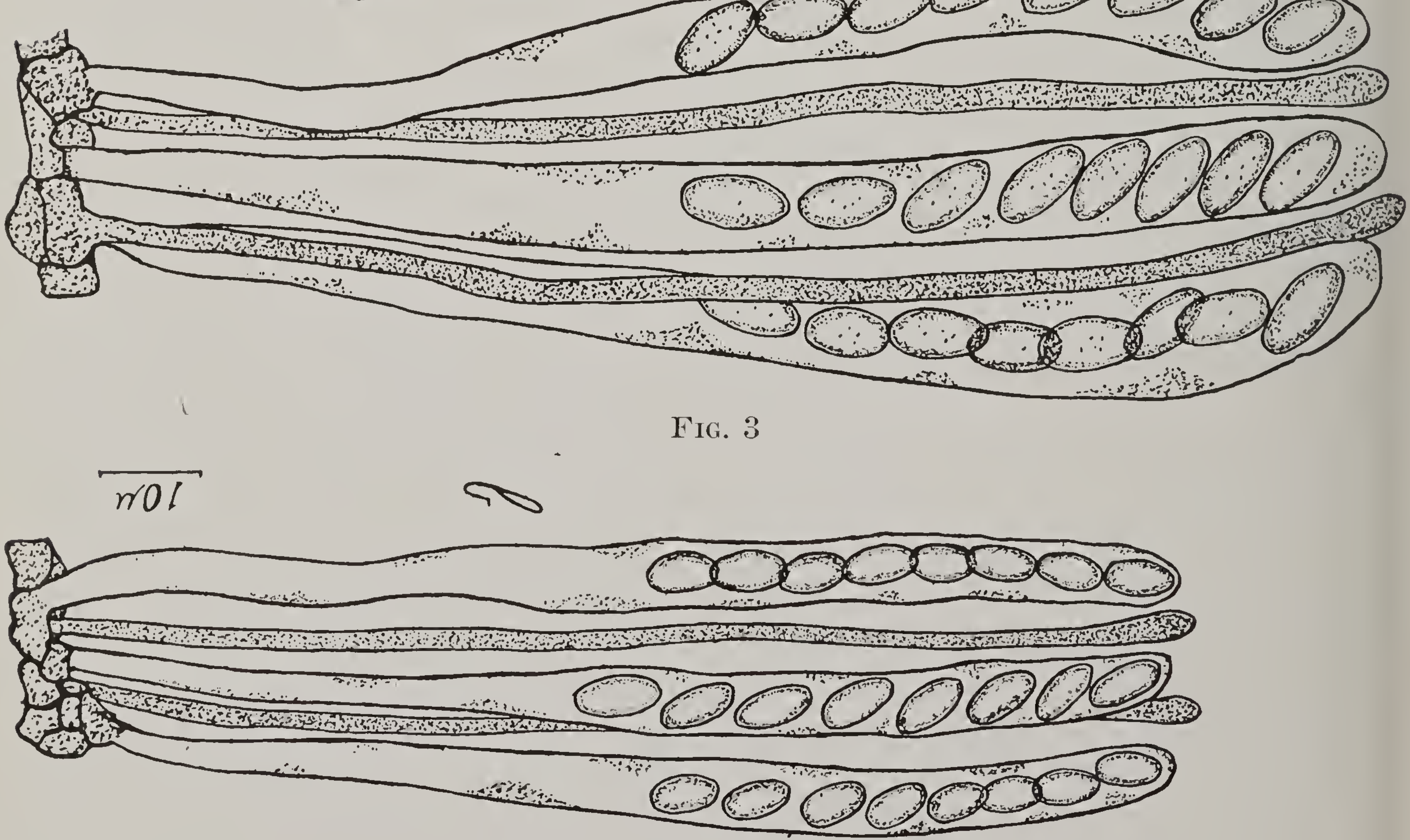

Fig. 3

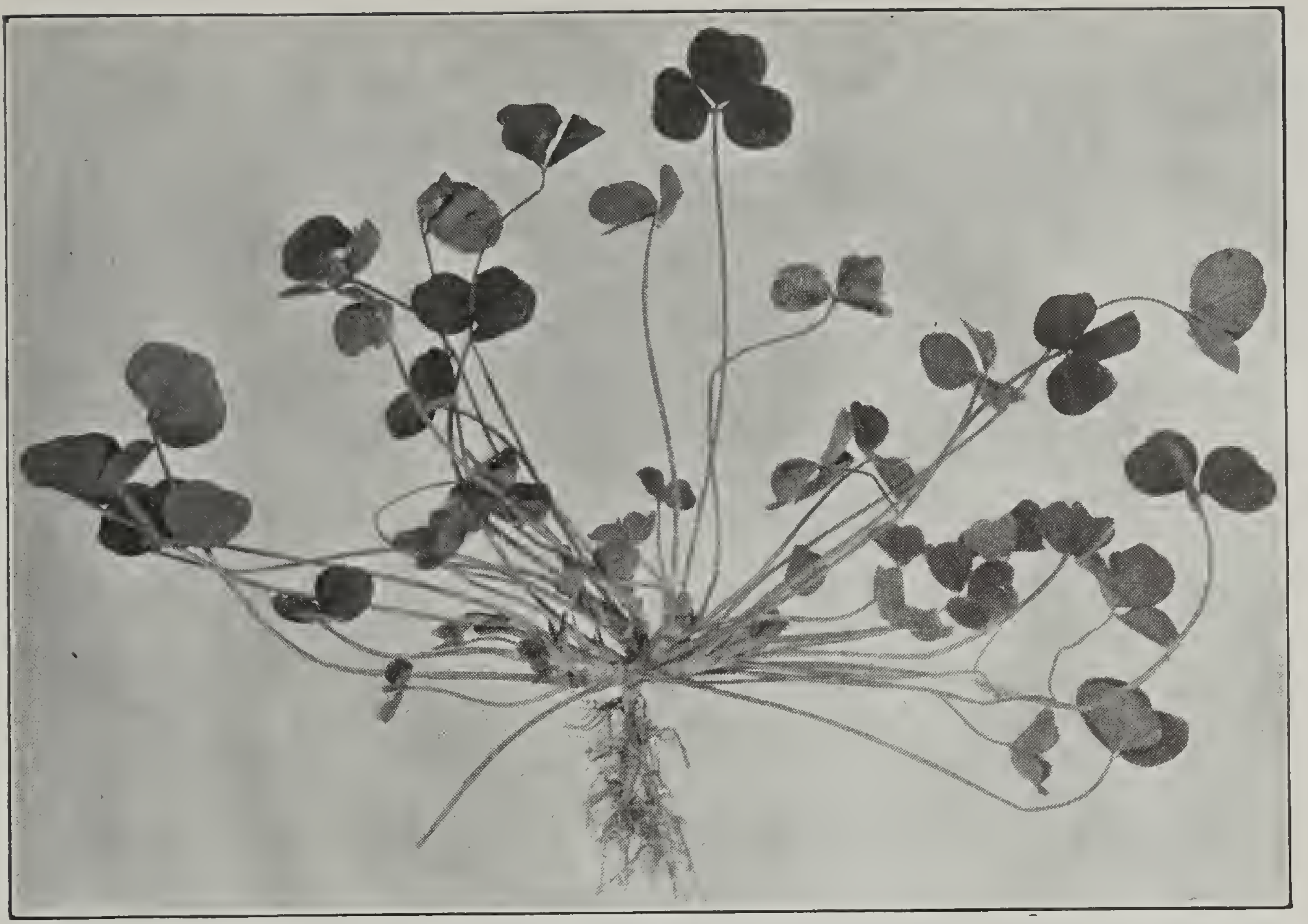

Fig. 1

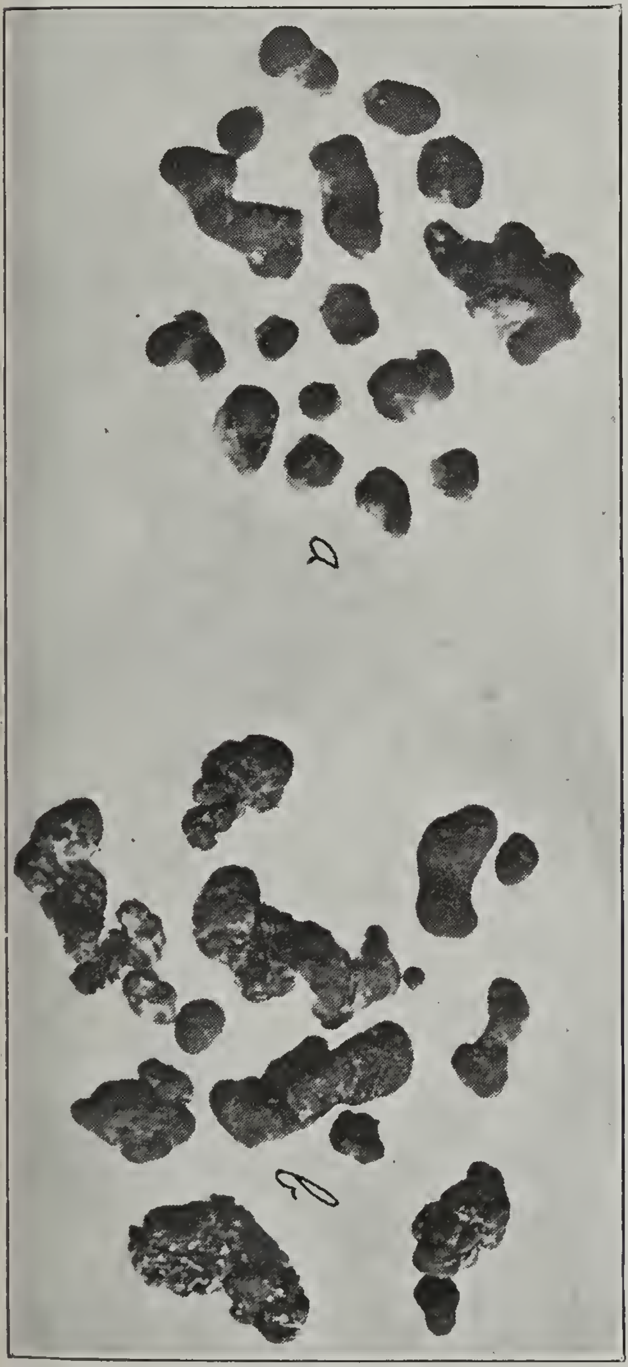

Fig. 2

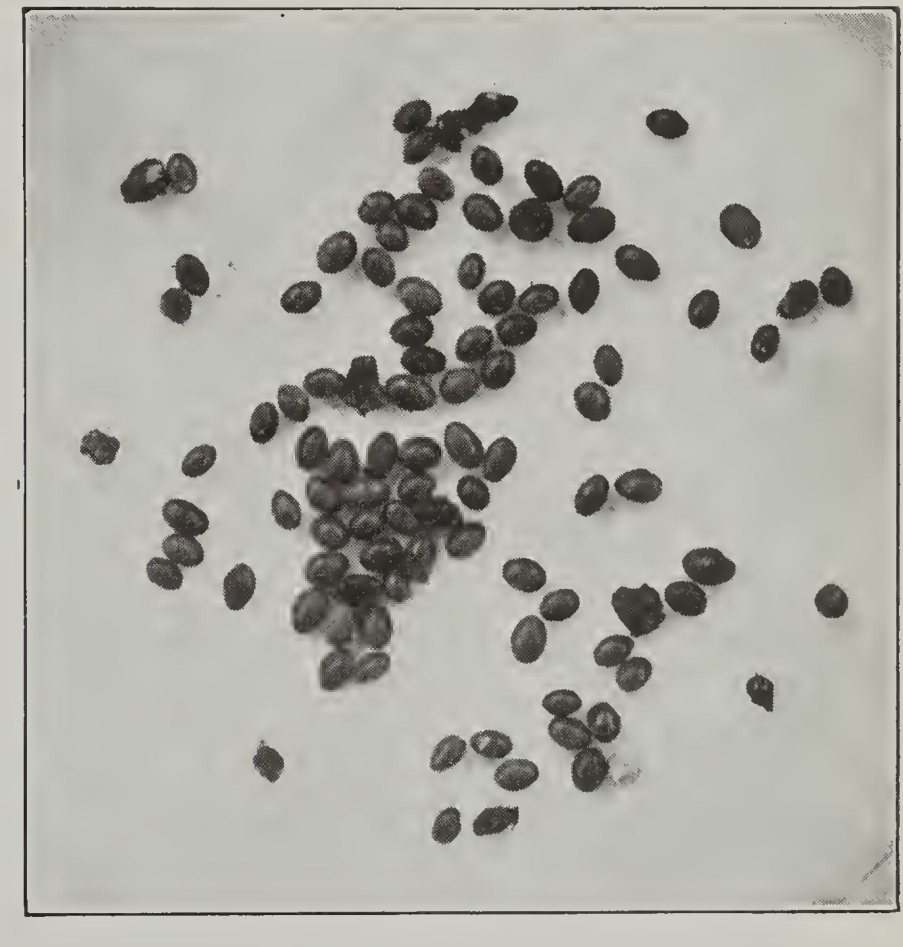

Fig. 3

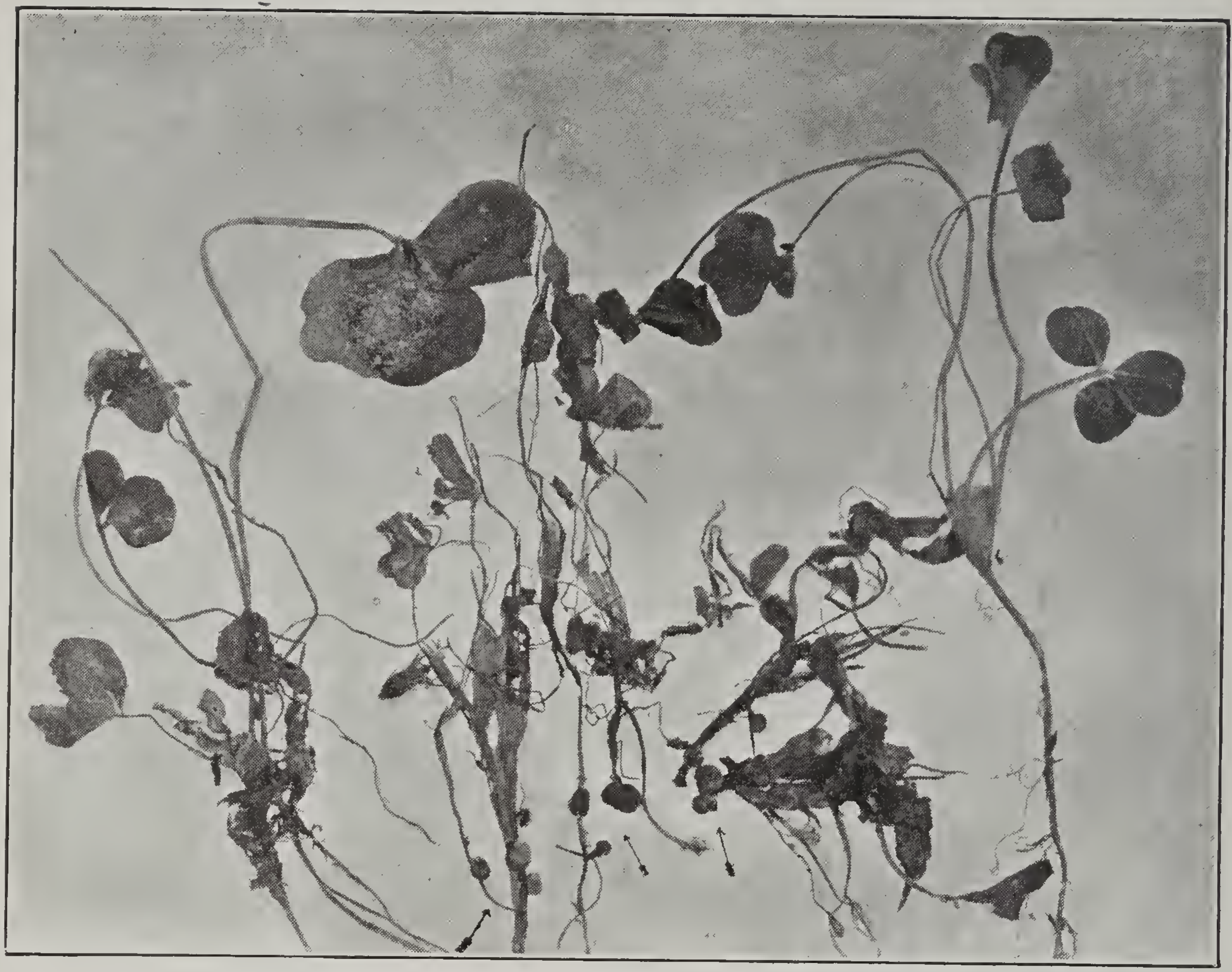

Fig. 1

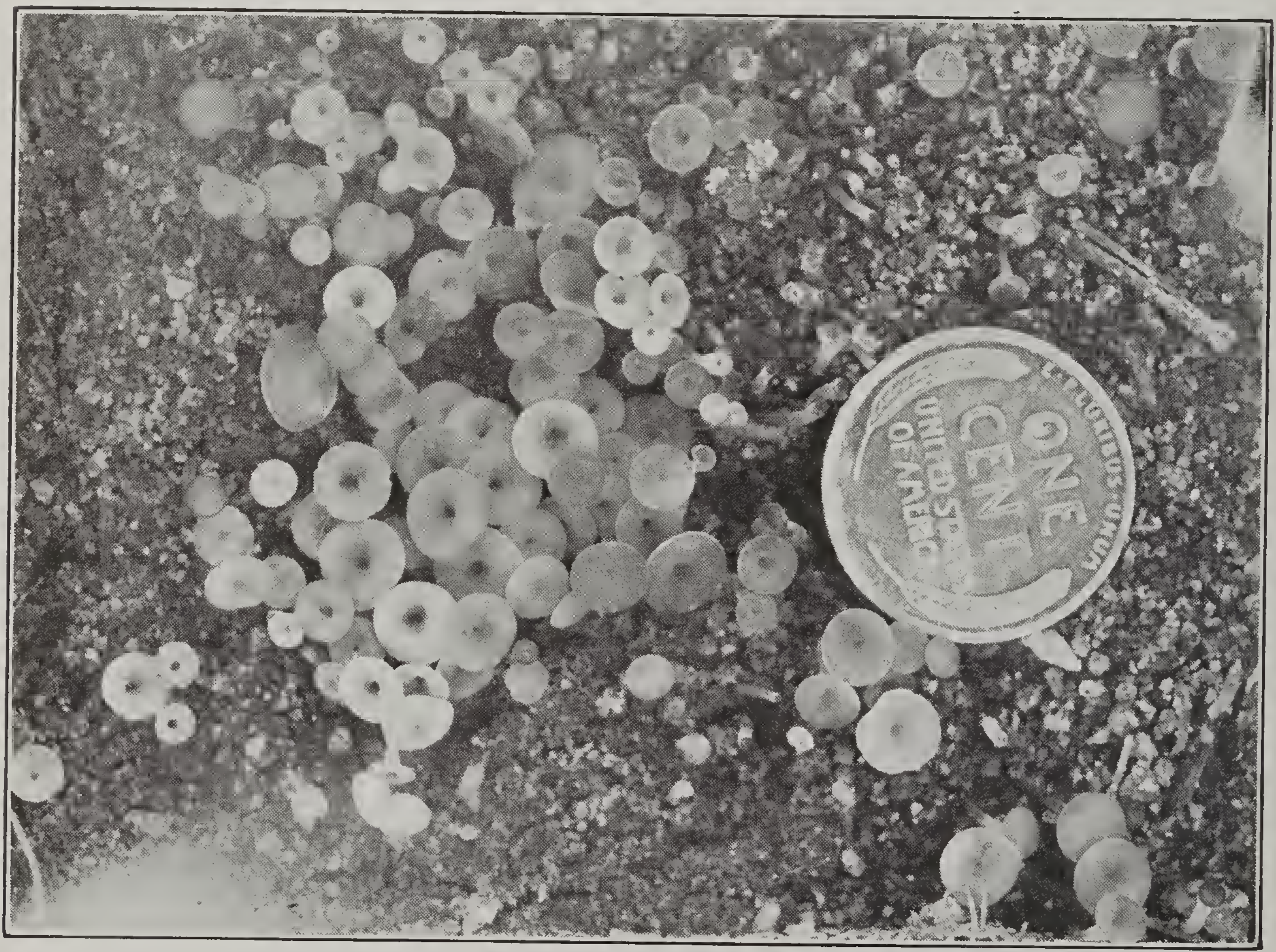

Fig. 2

R

JULY 1919 TECHNICAL BULLETIN No. 17

The Limits in Hybridization of Vitis Rotundifolia With Related Species and Genera

L. R. DETJEN
Division of Horticulture

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
CONDUCTED JOINTLY BY THE
STATE DEPARTMENT OF AGRICULTURE
AND THE
NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING
RALEIGH AND WEST RALEIGH

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THE NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

CONDUCTED JOINTLY BY THE

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THE LIMITS IN HYBRIDIZATION OF VITIS ROTUNDIFOLIA WITH RELATED SPECIES AND GENERA

FIRST REPORT*

L. R. Detjen, Assistant Horticulturist

INTRODUCTION

The investigational work with Muscadine grapes at the North Carolina Agricultural Experiment Station has proceeded to a point where it has become both desirable and necessary to determine at least the approximate limits within which hybridization of individuals of this species with other species, and even genera, can be effected. Such a determination being made, there is no doubt that conclusions concerning the most profitable course to pursue in the work of hybridization can be drawn, thus gaining valuable information and time in the work of improving this most important Southern grape.

Some work along this line has already been done, but because of the meagre amount of literature on the subject and its evident lack of support in the form of results of systematic investigation, as well as for the sake of collecting and preserving the facts concerning what has been accomplished, a brief review of the work of earlier grape breeders with special regard to the limits within which Vitis rotundifolia can be hybridized will first be made.

A REVIEW OF THE WORK ON HYBRIDIZATION OF VITIS ROTUNDIFOLIA

1. Work of Van Buren, Clarksville, Ga., Prior to 1868.

From all that we can learn of Van Buren's work in hybridizing Vitis rotundifolia, we find that he never was successful in producing a hybrid of this with any other species.

2. Work of Dr. Peter Wylie, Chester, S. C., Prior to 1877.

From the meagre evidence that is available of Dr. Peter Wylie's work on the hybridization of the Rotundifolia grapes, we learn that he was without doubt the first to produce hybrids with Vitis rotundifolia. These hybrids were the progeny of Vitis vinifera crossed with pollen from Vitis rotundifolia.

Because of the brevity of the article in which Dr. Wylie sets forth his conclusions drawn from work that he did with Vitis rotundifolia, the last two paragraphs from his report on progress of his experiments in hybridizing this species of grape found on page 116 of the Am. Pom. Soc. Report for 1871 are herewith reproduced.

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* Original manuscript submitted for publication January, 1919.

> My experience will go far to establish the following facts, viz.: that we cannot fertilize the Scuppernong with the pollen from any other species, or their hybrid varieties, or with male (staminate) hybrid Scuppernongs. Second, that we can impregnate the Foreign, (*vitis vinifera,*) with pollen from the Scuppernong, producing thereby only male (staminate) plants, and imperfect hermaphrodite or pistillate plants, which bear no fruit. Third, that we cannot impregnate Labrusca, Æstivalis, Cordifolia, or their hybrids with Foreign (*vitis vinifera,*) with pollen from Scuppernong. Fourth, that we can fertilize both native and foreign and their hybrids, with male (staminate) hybrid Scuppernong pollen, producing thereby prolific hybrid Scuppernong vines. Fifth, that we can fertilize those prolific hybrid Scuppernong plants, with pollen from the hybrid male (staminate) Scuppernong vines, thereby giving more of the Scuppernong constitution to the progeny. I find that the seed of the prolific hybrid Scuppernongs grow, and that you can impregnate other varieties with pollen from prolific hybrid Scuppernongs.
>
> Owing to various circumstances, which I will not attempt to detail, I have not pushed the production of prolific hybrid Scuppernongs, as rapidly as I expected and desired. There is one difficulty in using the hybrid Scuppernong pollen, where we have no hot-house; it blooms, like the Scuppernong, after nearly every other grape has done blooming; but, besides this difficulty, I have lost many plants and seed already hybridized, from other sources.

Without a doubt but rather unfortunately, Dr. Wylie uses the term Scuppernong as a specific (quite customary in his day and not even now completely eradicated from the vocabulary of the layman) instead of a varietal name as we find it in our present-day nomenclature. By keeping this terminology clearly in mind, the five conclusions reached by Dr. Wylie seem not as hazy and contradictory as might appear at first glance. By substituting the words Vitis rotundifolia in place of his term Scuppernong, these conclusions will read as follows: "First, that we cannot fertilize Vitis rotundifolia with the pollen from any other species, or their hybrid varieties, or with male (staminate) hybrid Rotundifolia vines. Second, that we can impregnate the foreign, (*vitis vinifera,*) with pollen from Vitis rotundifolia, producing thereby only male (staminate) plants, and imperfect hermaphrodite or pistillate plants, which bear no fruit. Third, that we cannot impregnate Labrusca, Æstivalis, Cordifolia, or their hybrids with Foreign (*vitis vinifera,*) with pollen from Vitis rotundifolia. Fourth, that we can fertilize both native and foreign and their hybrids, with male (staminate) hybrid Rotundifolia pollen, producing thereby prolific hybrid Rotundifolia vines. Fifth, that we can fertilize those prolific hybrid Rotundifolia plants, with pollen from the hybrid male (staminate) Rotundifolia vines, thereby giving more of the Rotundifolia constitution to the progeny. I find that the seed of the prolific hybrid Rotundifolia vines grow, and that you can impregnate other varieties with pollen from prolific hybrid Rotundifolia vines."

In the second conclusion, we see where Dr. Wylie successfully hybridized Vitis vinifera with pollen from Vitis rotundifolia. From the hybrid seeds he grew progeny which consisted of two types, namely: male (staminate) plants and imperfect hermaphrodite plants which bore no fruit.

Although these hybrid vines are nowhere described, any doubt as to the authenticity of their hybrid nature is soon dispelled when we read that the imperfect hermaphroditic plants bear no fruit. This means that they were practically sterile. From our work with hybrids of this nature to be published in another bulletin, we have observed that this sterility is very pronounced and typical in the F_1 hybrids and such sterility should not occur if his seedlings were not true hybrids. Furthermore, in his last paragraph, Dr. Wylie clearly states that the blooming period of these hybrid vines is like that of the "Scuppernong" (meaning V. rotundifolia) which is later than that of nearly every other species. With us V. rotundifolia begins to bloom fully two weeks later than V. vinifera, and some of the hybrids also bloom at a similarly late date. Unfortunately these Rotundifolia hybrid vines of Dr. Wylie were lost to horticulture during a period of misfortune that befell that great Grape Breeder.

From this data, meagre as it is, the evidence is quite clear that Dr. Wylie must be recognized as having been the first to successfully hybridize Vitis vinifera with Vitis rotundifolia pollen.

Dr. Wylie failed completely to hybridize Vitis rotundifolia with pollen from any species of Euvitis, their hybrids, and even from his own male (staminate) hybrid Rotundifolia vines. He also failed completely to hybridize V. labrusca, V. æstivalis, V. cordifilia or any other native species of Vitis or their hybrids with V. vinifera, with pollen from V. rotundifolia. It seems his conclusions were based on insufficient experimental evidence because many such hybrids have since been produced.

From the very nature of the sterility of the F_1 hybrids with species of Euvitis, and also from the fact that hermaphroditic varieties are frequently subject to self-fertilization in the bud, Beach* (1), M. J. Dorsey (2), Hedrick and Anthony (3), it seems rather doubtful whether Dr. Wylie actually produced the attenuated (prolific) hybrids as he states in his fourth and fifth conclusions.

This resumé practically covers the most important part of Dr. Wylie's work in the hybridization of Vitis rotundifolia with related species and genera. He performed much work and attempted to establish and formulate limits beyond which hybridization cannot be effected, but fell far short of attaining that end. His conclusions are, therefore, subject to revision.

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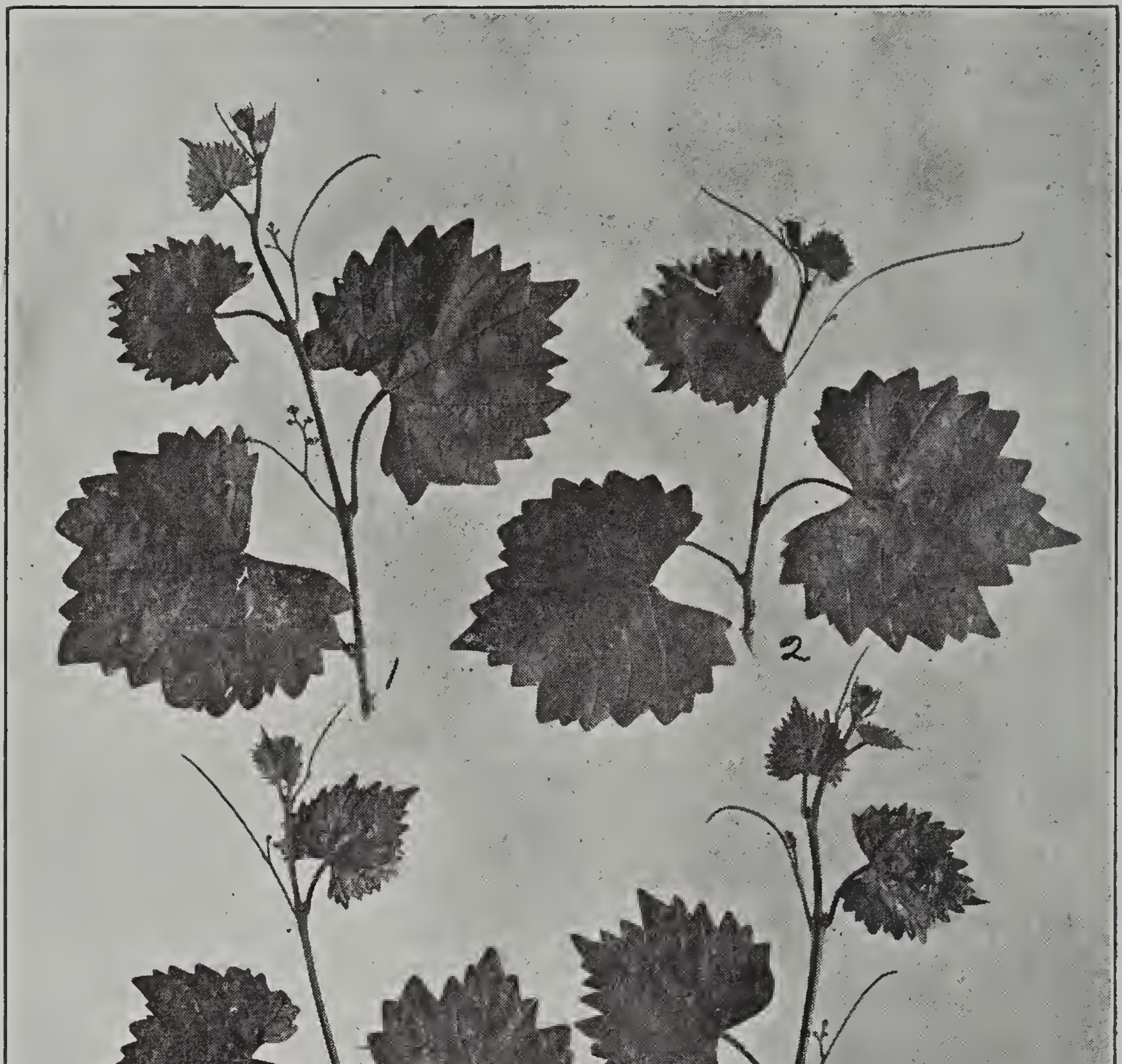
* References to cited literature will be made by number.



Six fruiting branches of as many vines which Dr. Munson placed on the market as being hybrids between V. rotundifolia and species of Euvitis. The six varieties are known as follows: (1) Sanalba, (2) Labama, (3) La Salle, (4) San Jacinto, (5) San Melaska, (6) Sanmonta. Reduced.

3. WORK OF PROFESSOR A. MILLARDET, FRANCE, SUBSEQUENT TO 1877.

All we can learn of the work of Prof. A. Millardet on the hybridization of the Rotundifolia grape is that he never was successful in its hybridization. He produced a lot of seedling vines which he fully believed were true hybrids but which we shall later learn were apparently only direct descendants of Vitis rotundifolia.

4. WORK OF DR. T. V. MUNSON, DENISON, TEXAS, 1890-1900.

In 1891 Dr. T. V. Munson saved seed from fruits of a Scuppernong vine which he presumed to have been pollinated and fertilized with pollen from some near-by Post-Oak x Herbemont hybrid vines. From the resulting seedlings of this lot he saved some 50 vines which he considered as hybrids, for a trial in his nursery. From these 50 seedlings he finally selected two which he named La Salle and San Jacinto and these he propagated and disseminated as his first hybrid varieties.

In 1898 Dr. Munson covered some flower clusters on the San Jacinto vine with tissue paper bags and pollinated these with pollen from Briliant, which is a Labrusca-Vinifera-Bourquiniana hybrid. From the seeds produced by these clusters he grew some 200 plants from which he selected 85 for trial in his nursery. From these he finally selected four vines for propagation and dissemination and named them Sanalba, Sanmelaska, Sanamonta and Sanrubra.

Dr. Munson also made a number of hybrids between Vitis rotundifolia and Vitis munsoniana, chief among which is the DeSoto.

With the exception of the last one, one vine of each of the above named varieties was secured by the North Carolina Experiment Station in January, 1909, and these plants have since that time been constantly under observation. After a time, the observations made gave rise to some doubt as to their real hybrid origin, and more recently to a very careful comparison of these vines with known hybrid material produced in the breeding plots at this Station. These comparisons together with conclusions drawn from them will appear in a separate bulletin which will follow immediately.

Although we regret it very much, suffice it to say here that while Dr. Munson firmly believed he had produced hybrids between V. rotundifolia and species of Euvitis and disseminated his choicest selected vines as such, these vines are not true hybrids but only seedling vines directly descended from the Muscadinia group.

5. WORK OF THE NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION 1911 AND 1912.

The North Carolina Agricultural Experiment Station began (4) its work in the hybridization of Vitis rotundifolia with related species in the spring of 1911. During this season, all of the work was confined to an effort to hybridize the variety Scuppernong with pollen from Vitis æstivalis, Vitis cinera, Vitis bourquiniana variety Herbemont and from the Labrusca-Vinifera hybrid variety Niagara. No hybrid fruits were secured from these crosses. The one seedling vine that was reported as having been secured from the Scuppernong-Herbemont cross proved itself in later years to be only a straight seedling of Vitis rotundifolia.

In 1912 the effort to hybridize the variety Scuppernong was repeated. Scuppernong flowers were again dusted with pollen from Vitis æstivalis, Vitis bourquiniana variety Herbemont and from the Vinifera-Labrusca-Æstivalis hybrid variety Winchell. No hybrid fruits were secured from this work.

Two other crosses, Herbemont and Winchell varieties crossed with pollen from a light-colored staminate vine of Vitis rotundifolia, were made during this season but were not reported because no definite conclusions had been reached in regard to their character up to the time of publishing the last report.

The conclusions drawn in 1914 were to the effect that the variety Scuppernong would not readily hybridize with pollen from V. æstivalis, V. cinerea, V. bourquiniana var. Herbemont and the hybrid varieties Niagara and Winchell. These conclusions have been substantiated by the more extensive work of subsequent years.

6. WORK OF THE VITICULTURAL DIVISION OF THE U. S. DEPARTMENT OF AGRICULTURE, 1911-1917.

The Viticultural Division of the U. S. Department of Agriculture began its work on the hybridization of Vitis rotundifolia with species of Euvitis in 1911. A brief discussion of this work follows.

In 1911 the efforts of hybridizing V. rotundifolia were reported as having resulted in a complete failure.

In 1912 one supposed hybrid was secured, a cross between the Rotundifolia Munsoniana hybrid variety Eden as the female parent and V. vinfera variety Flame Tokay as the pollen parent. This supposed hybrid is described thus, "While it is distinct from other Muscadine seedlings of Eden parentage, it is predominantly Muscadine in character and shows no resemblance to Flame Tokay except in leaves." From the description of this vine and with a fairly good knowledge of the character of these hybrids in general, we should assume it to be probable that this supposed hybrid is similar to those produced by Professor A. Millardet of France and those produced later by Dr. T. V. Munson of Denison, Texas, which will be found discussed under the caption "False Hybrids" in the succeeding bulletin on "Some F_1 Hybrids between Vitis Rotundifolia and Related Species."

The Department reports that in 1913 twenty-two hybrids were produced, of which only eight survived the vicissitudes of the seed bed.

Seven of these surviving vines are said to be of Olivette de Vendemain parentage; no mention is made of the other parent vine. The offspring of this Olivette de Vendemain are described as "Variable in vigor and character, some being vigorous and others weak; some are more Vinifera in type and others more Muscadine." The eighth seedling was the result of crossing the variety Scuppernong with pollen from the variety Winchell. This seedling is reported as being the only hybrid that has been secured by the Department from Scuppernong parentage. It is described thus: "It resembles Winchell more than Scuppernong, though from a Scuppernong seed." The description of this vine, meagre as it is, when compared with the description of a similar but reverse cross, Winchell x Rotundifolia produced by the North Carolina Agricultural Experiment Station in 1912 and which will be found described in the succeeding bulletin, seems sufficient to warrant calling this seedling a true hybrid.

The Department reports that in 1914 the following hybrids were produced and now are growing at Willard, N. C.:

2 seedlings of Eden × Maravilla de Malaga.
1 seedling of Thomas × Rodites.
3 seedlings of Thomas × Carignane.
1 seedling of Thomas × Noah.
1 seedling of V16 R6 B2 × Carignane.
3 seedlings of V17 R6 B2 × Terret Monstre.

In 1915 seventy hybrid seedlings were produced which are represented by the following Euvitis varieties:

Muscat of Alexander	(V. vinifera)
Calabrian	(V. vinifera)
Ferrara	(V. vinifera)
Rodites	(V. vinifera)
Semillon	(V. vinifera)
White Hanepoot	(V. vinifera)
Prune de Cazouls	(V. vinifera)
Huasco	(V. vinifera)
Winchell	(Labrusca-Vinifera-Æstivalis)
Goethe	(Vinifera-Labrusca)
Brilliant	(Labrusca-Vinifera-Bourquiniana)
Catawba	(Labrusca-Vinifera)
Iona	(Labrusca-Vinifera)
Ives	(Labrusca-Æstivalis)

In 1916 a larger crop of hybrid seeds were secured and many seedlings are reported to be growing.

In all of the hybridization work, only imperfect hermaphroditic Muscadine vines were used as the female parents because of their diœciousness, and reciprocal crosses apparently were not attempted.

The Department reports that of the commercial varieties, Thomas seems the most congenial to the pollen of Euvitis varieties, although some Muscadine seedlings seem even more promising for work in hybridization.

It will be noticed from the foregoing that the given varieties of Euvitis will easily fall into two categories; namely, those of the pure species of Vitis vinifera, and those that are of hybrid origin. Thus only one pure species, Vitis vinifera, is represented in all of this work and all of the other Euvitis varieties, with only one exception (Ives), also contain some blood of the Vitis vinifera species.

While these results represent a large amount of work and are of much practical importance and value, yet that broader field which pertains to different species and genera and reciprocal crosses seems to have been entirely neglected. Results secured from hybrid varieties always will represent what can be done with that or a similar variety and no more, except where bridging becomes a necessity.

This concludes the review of the work on the hybridization of Vitis rotundifolia with related species as has been reported from time to time in Horticultural literature.

WORK DONE AT THE NORTH CAROLINA EXPERIMENT STATION 1912-1918 INCLUSIVE

1. Scope of the Work in Hybridization.

From a commercial point of view, the Rotundifolia grapes have proven their worthiness of a place in our permanent national horticulture. The layman has begun to appreciate the real economic value of the vine and his demands for the products of this grape are large and ever on the increase. As never before people all over the world are looking upon Vitis rotundifolia as a new, distinct and valuable asset to our viticulture.

Although Vitis rotundifolia is so valuable and so full of greater promise, it is defective notably in the unimproved state of the vine and natural undesirable characters of the fruit. The former is easily overcome, but the latter is difficult to eradicate. Improvement has become the pass-word among viticulturists. There are, however, many ways of improving a plant. Hybridization is one of these and a fruitful source of variation. The scope and object of this article will be an attempt to discuss, in part, the limits of hybridization of Vitis rotundifolia with related species and genera. By knowing the limits in hybridization and the comparative ease or difficulty with which hybrids of known parentage can be secured, much unnecessary work and vexation may be spared the practical grape breeder.

2. MATERIALS AND METHODS USED.

In order to study the limits in hybridization of a particular species of plant, it becomes imperative to collect for study and use as many of its allied species, genera and hybrids as is practicable. Consequently, we have made a collection of as many of the varieties and species of Vitis and hybrids with Vitis rotundifolia and some species of Parthenocissus and Ampelopsis as were possible to obtain and grow under our local conditions. Many of the species come from distant States, but most of these so far studied and to be reported on in this bulletin with the exception of Vitis vinifera and named varieties of other species are native vines of local habitat. The behavior of other species in hybridization toward Vitis rotundifolia will be reported on in future publications.

The varieties, species and genera reported on in this bulletin are as follows:

(a) Vitis rotundifolia.
(b) Rotundifolia-Munsoniana hybrids.
(c) Vinifera-Labrusca-Æstivalis hybrid, variety Winchell.
(d) Vitis bourquiniana, variety Herbemont.
(e) Vitis vinifera, variety Malaga and two of its seedlings.
(f) Vitis labrusca, native species and the two varieties, Concord and Lutie.
(g) Vitis cordifolia, native species.
(h) Vitis cinerea, native species.
(i) Vitis æstivalis, native species.
(j) Parthenocissus quinquefolia (Planch.), native species (Virginia Creeper).
(k) Parthenocissus tricuspidata (Planch.) (Boston Ivy).
(l) Ampelopsis heterophylla (Sieb. & Zucc.), variety Elegans (Koch) (Variegated Ivy).

The methods used in all of the hybridization work are those that are commonly in vogue (See also Breeding Southern Grapes, Journal of Heredity, Vol. VIII, No. 6). The bags employed for this work were made of what is commercially known as Pacific Lawn.

3. RESULTS SECURED FROM 1912 TO 1918, INCLUSIVE.

Vinifera-Labrusca-Æstivalis Hybrid Var. Winchell × Vitis Rotundifolia

In 1912 Winchell (Vinifera-Labrusca-Æstivalis) vines were used as the female parent and a light-colored male vine (Rotundifolia) was used as the pollen parent. Out of 99 seeds, 34 plants were secured, but as the female parent vines were of a hermaphroditic and self-fertile variety, the number of hybrid vines in this lot was subject to some doubt. Because of the poor conditions existing in the seed-bed, most of the seedlings of this cross perished. Eleven vines were finally transplanted to the nursery for study. Later on it developed that ten of these eleven seedling vines were direct selfed descendants of Winchell, but one of the eleven was a true hybrid. This hybrid plant is living and healthy at this date. This is the first true Rotundifolia hybrid with the native Euvitis grape (although of hybrid origin) that is recorded. It will be found fully described in our succeeding bulletin entitled, "Some F_1 Hybrids Between Vitis Rotundifolia and Related Species." In the spring of 1916 the variety Winchell was again used as the female parent plant and two self-fertile seedlings (Rotundifolia) were used as the male parent.

The first cross, Winchell x I-1 (light colored seedling of Scuppernong x Hope), resulted in 378 seeds. Of these, 79 hybrid seedlings that were strong enough to set in the nursery were obtained.

The second cross, Winchell x G-52 (dark colored seedling of Thomas x Hope), resulted in 34 seeds. Among the resultant seedlings from this lot, only one strong hybrid was obtained and transplanted to the nursery.

Vitis Rotundifolia × (Vinifera-Labrusca-Æstivalis Hybrid) Var. Winchell

In order to test the possibility of a reciprocal cross (V. rotundifolia x Winchell), two Rotundifolia seedlings X-32, a seedling of Scuppernong crossed with Light Male No. 2, and a dark selfed seedling of Hope were used in 1916. The first, an imperfect hermaphroditic vine (X-32), had ten bags covering twenty flower clusters. The flowers on these clusters were hand pollinated every morning with fresh Winchell pollen. None of the flowers developed fruit.

The dark seedling of Hope, a perfect flowering vine, had only one flower cluster prepared and it was crossed with Winchell pollen. No fruit set on this cluster.

Thus far our effort to hybridize Vitis rotundifolia with Winchell pollen has met with only negative results. Dearing (5) reports the production of some hybrid vines from Vitis rotundifolia crossed with Winchell as the male parent. It is quite apparent that hybrids of this reciprocal cross are rather difficult to obtain.

Vitis Bourquiniana Var. Herbemont × Vitis Rotundifolia

In 1912 Vitis bourquiniana var. Herbemont was used as the female parent and crossed with V. rotundifolia, Light Male vine No. 2. In the fall of the year 28 seeds were harvested. Of these 28 seeds only two germinated and these died soon afterward.

In 1913 Herbemont was crossed with V. rotundifolia, Light Male vine No. 1, as the pollen parent. Four clusters of the Herbemont vine were emasculated and later crossed with pollen from the male vine. In the fall 19 seeds were gathered from this cross and out of this lot of seeds in 1914 one true hybrid vine, strong enough to withstand the vicissitudes of the seedbed, was secured. This hybrid vine is living today and will be fully discussed in our succeeding bulletin. This is the first Herbemont-Rotundifolia hybrid that was ever produced and recorded.

In 1916 and 1917 Herbemont was again crossed with V. rotundifolia, G-52 being the male parent. Five flower clusters on the Herbemont vine were prepared in 1916 and later cross-pollinated with pollen from G-52. A total of 35 seeds was harvested and from this lot of seed 7 fairly strong hybrid vines were secured. Seven flower clusters on the Herbemont vine were prepared in 1917 and again cross-pollinated with pollen from G-52. A total of 86 seeds was harvested from this cross but only 5 fairly strong hybrid vines were secured. Of these two lots of hybrid vines only seven vines are at present (1919) alive. Most of the hybrids of Herbemont with V. rotundifolia are constitutionally weak and only a comparatively few seem able to survive.

Vitis Rotundifolia × *Vitis Bourquianiana*

The results of the reciprocal cross V. rotundifolia x V. bourquiniana var. Herbemont have been altogether negative thus far. In 1916 two Rotundifolia seedlings, X-32 and a perfect flowered selfed seedling of Hope, were used. Ten bags covering 20 flower clusters were applied to the vine X-32 and four bags covering as many flower clusters were applied to the perfect flowered seedling of Hope. The expanding flowers were in due season dusted with fresh pollen from the variety Herbemont. No fruits were secured from either of these pollinations.

In 1917 three flower clusters on a Scuppernong vine* which had been forced in the greenhouse were bagged and cross-pollinated with Herbemont pollen. During the same season seven flower clusters on a Scuppernong vine in the vineyard were bagged and cross-pollinated with Herbemont pollen. N-28 and O-23, two seedlings of Scuppernong x Dark Male vine No. 1 had one flower cluster each bagged and crossed with Herbemont pollen. E-19, a seedling of Thomas x Light Male vine No. 2 also had two flower clusters treated in the same manner. Not one berry was produced in all of these crosses.

Rotundifolia-Munsoniana Hybrid × *Vitis Bourquiniana*

In 1917 three flower clusters of the variety Eden, a Rotundifolia-Munsoniana hybrid, were bagged and cross-pollinated with Herbemont pollen. No fruit was secured from this cross.

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* This Scuppernong vine was grown in a box and some time during the month of February was placed in the greenhouse for forcing. In order to ascertain whether this forcing might exert any evil influences in regard to the production of fruit, one flower cluster was cross-pollinated with normal Rotundifolia pollen and typical fruit was obtained. The vine, therefore, was in proper condition to enter into the experimental work.

To obtain this reciprocal cross is apparently a much greater task than to obtain the original cross where Herbemont is used as the female parent.

Vitis Vinifera × *Vitis Rotundifolia*

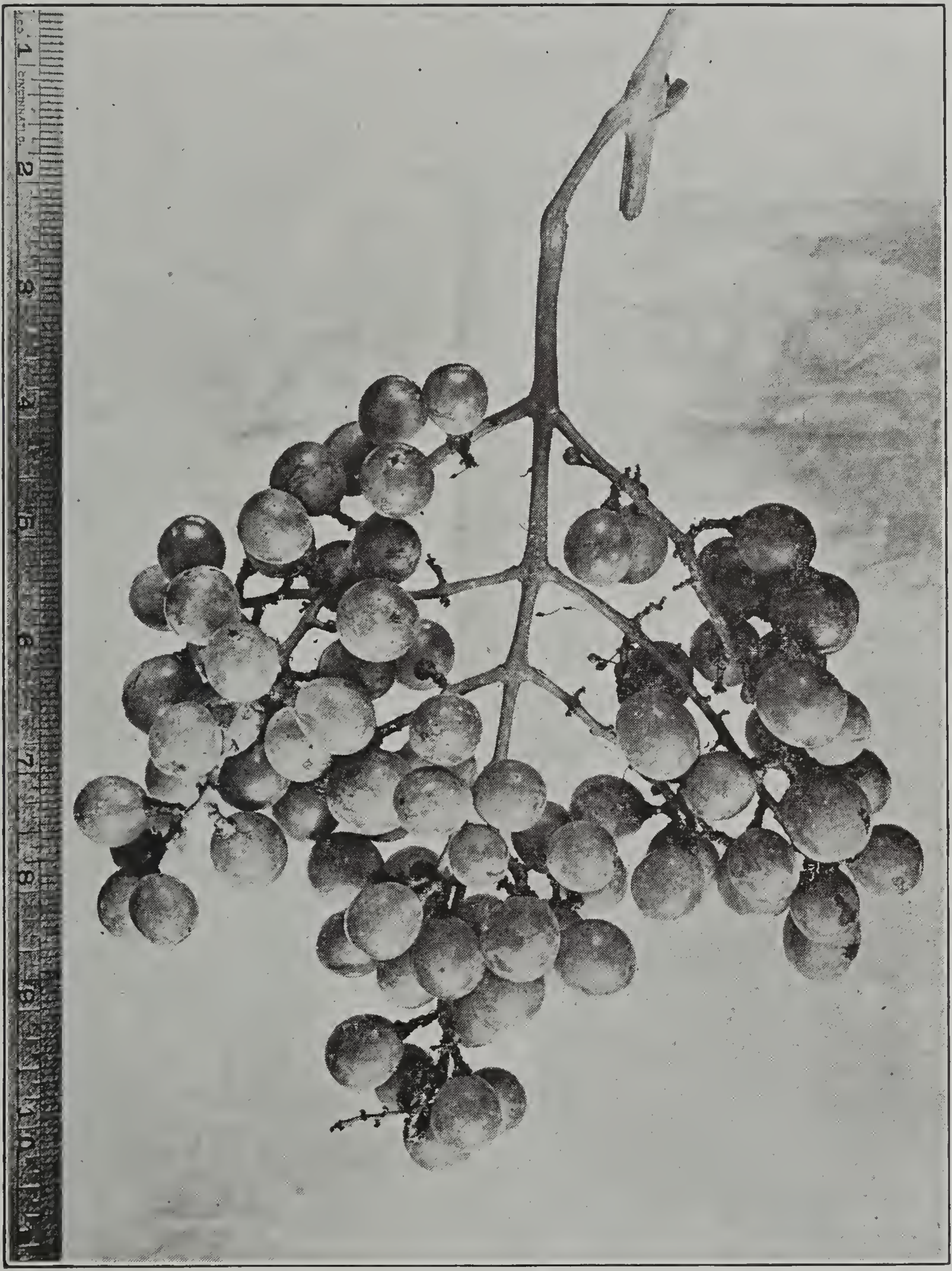
In 1916 three flower clusters on an imperfect hermaphroditic Vinifera vine, Malaga Seedling No. 1, were prepared, covered and later cross-pollinated with pollen from the Rotundifolia vine I-1. Four similar flower clusters were cross-pollinated with pollen from G-52. In the fall 16 seeds were obtained from the cross with I-1 and 15 seeds from the cross with G-52. All the seeds grew in 1917 and 26 were set in the nursery for study.

During this same season (1916) Malaga Seedling No. 1, as well as Malaga Seedling No. 3 which will be found mentioned later, have been thoroughly tested for any signs of self-fertility, and both vines have proven to be practically incapable of producing seed by themselves. Knowledge of this sort is of tremendous importance for the breeder because much labor, time and material can be saved by circumventing the process of emasculating the flowers. The results obtained with Malaga Seedling No. 3 in 1917 amply justify this contention.

In 1917 this seedling was cross-pollinated with pollen

A Vinifera-Rotundifolia seedling vine two years of age with eight of its flower clusters bagged for a test in self-fertility. Reduced.

from I-1. Six bags were used and as many clusters treated. These flowers were not emasculated as were the preceding ones, but had the foreign pollen administered to them as soon as they had opened. From



A fruit cluster from Malaga Seedling No. 3, whose self-sterile flowers had been bagged and treated to pollen from a Rotundifolia vine, showing how readily some Vinifera varieties will produce fruit with pollen from V. rotundifolia. Reduced.

this cross 218 seeds were obtained and of the resulting hybrid seedlings 52 were set in the nursery. Four of these died from drought during the summer of 1918.

In 1917 Malaga also was used as a female parent. Four flower clusters were cross-pollinated with I-1 and eight similar clusters were cross-pollinated with G-52. From these covered and cross-pollinated clusters 93 and 143 seeds respectively were obtained.

Because of limited available space in the nursery in 1918, only 14 true hybrids of the Malaga x G-52 cross were retained and none of the seedlings of the Malaga x I-1 cross were planted.

Vitis Rotundifolia × Vitis Vinifera

In 1917 one flower cluster on the forced Scuppernong vine in the greenhouse was bagged and cross-pollinated with pollen from Malaga. No fruit developed.

The same year W-38, a seedling of Scuppernong x Dark Male No. 1, was used as the female parent and Malaga was used as the male parent. Seven flower clusters on W-38 were bagged and pollinated with fresh pollen from the Malaga vine. One fruit developed from this work, but not as the result of hybridization.

Five flower clusters on G-12, an imperfect hermaphroditic seedling of Thomas x Hope, were bagged and pollinated with Malaga pollen. No fruit resulted.

Five flower clusters on the Flowers vine were bagged and pollinated with Malaga pollen. No fruit developed from this work.

Ten flower clusters on the Oberlin vine, a Rotundifolia variety originally found growing wild in Oberlin and only about one mile from the college campus, were bagged and pollinated with Malaga pollen. From this work three berries developed and ripened, from which five seeds were secured. These seeds were planted and in 1918 two true hybrid vines were obtained. These two vines are still growing and will be more fully discussed in our succeeding bulletin.

In 1918 ten flower clusters on the Oberlin vine were again bagged and pollinated with Malaga pollen, but no fruit developed from this work.

From the results of the preceding efforts we find that the reciprocal cross, Vitis rotundifolia x Vitis vinifera, although difficult to obtain can be effected. Dearing (5) finds certain varieties of both species more congenial to hybridization than others.

Vitis Labrusca × Vitis Rotundifolia

In 1917 ten flower clusters on a Concord vine (V. labrusca) were prepared, bagged and pollinated with pollen from G-52. In the fall of the year 130 seeds were obtained and planted. From this lot of seeds 12 true hybrid seedlings were observed to grow; others may have germinated but were not recognized as such before their death. Only five of the hybrids were strong enough to be transferred to the nursery.

Ten flower clusters on a native imperfect hermaphroditic vine (V.

labrusca) were bagged in 1917 and dusted with pollen from G-52. From this work no fruit was obtained.

In 1918 twenty-two flower clusters on the same native Labrusca vine were bagged and the flowers were dusted with pollen from I-1. In the fall of the year fruit was taken from seven of the bags and a total of 83 seeds was obtained. All of these seeds are the result of a cross because the mother vine which bears reflexed stamens has proven itself to be absolutely self-sterile.*

In 1918 eleven flower clusters on the variety Lutie (V. labrusca) were prepared, bagged and dusted with pollen from I-1. From this work 283 seeds have been obtained. These seeds will be planted in 1919 and the hybrid character of the seedlings noted.

Vitis Rotundifolia × *Vitis Labrusca*

In 1917 four flower clusters on I-1 were prepared, bagged and pollinated with Concord pollen. No fruits developed from this work.

In 1918 six flower clusters on a forced Scuppernong vine were bagged and pollinated with Lutie pollen. Again no fruits developed.

From the results of this work we again see a difficulty in securing the reciprocal cross, V. rotundifolia x V. labrusca.

Vitis Æstivalis × *Vitis Rotundifolia*

In 1918 nine flower clusters on a native imperfect hermaphroditic Æstivalis vine were bagged and cross-pollinated with pollen from I-1. In eight of these bags no fruit developed and in the ninth bag a small cluster of apparently normal fruit developed. This cluster yielded 20 seeds which will be planted, and the resulting seedlings will be keenly watched and examined for hybrid characters.†

Vitis Rotundifolia × *Vitis Æstivalis*

In 1917 one flower cluster on a seedling Rotundifolia vine, N-28, one on another seedling, K-19, and seven on Scuppernong were bagged and dusted with fresh pollen from a staminate Æstivalis vine. No fruits developed in any of these bags.

In 1918 eight flower clusters on Scuppernong and ten on the variety James were bagged and treated as before. The bags on the Scuppernong vine yielded no fruit while only one bag on the James vine yielded but two berries. It is to be regretted that this bag, together with its contents, disappeared just when the fruit was ripening on the vine.

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* Since the manuscript for this bulletin was prepared these seeds have germinated and after a careful examination of them all possible doubt as to their hybrid nature has been dispelled.

† Since the manuscript for this bulletin was written these seeds were planted and nineteen of them germinated. Most of these seedlings are constitutionally weak and thus far only nine have survived, but all of them have proved to be true hybrids with Vitis rotundifolia.

Rotundifolia-Munsoniana Hybrid × Vitis Æstivalis

In 1917 two flower clusters on the variety Eden were bagged and dusted with fresh Æstivalis pollen. No fruit developed in these bags.

In 1918 ten more flower clusters on the same Eden vine were bagged and treated as before. One fruit developed, from which one seed was obtained. This seed will be planted and the resulting seedling, if it survives, will be studied and duly examined for hybrid characters.

Vitis Cordifolia × Vitis Rotundifolia

In 1917 ten flower clusters on a native imperfect hermaphroditic Cordifolia vine were bagged and dusted with pollen from G-52. In the fall some fruit was harvested and a total of 12 seeds was obtained. These seeds were planted and five seedling vines resulted. Of these only two survived the seedbed and were set in the nursery. They will be described more fully in our succeeding bulletin.

In 1918 ten flower clusters were again bagged on the same Cordifolia vine and later dusted with pollen from I-1. In the fall fruit was harvested from each of the bags and a total of 174 seeds was obtained. These seeds will be planted and the resulting seedlings will be duly cared for.

Vitis Rotundifolia × Vitis Cordifolia

In 1917 fourteen flower clusters on a Scuppernong vine were bagged and dusted with pollen from a staminate Cordifolia vine. No fruit developed from this work. In 1918 eight flower clusters were again bagged on a Scuppernong vine and dusted with pollen from a staminate Cordifolia vine. Again no fruit developed. Here we once more encounter difficulty in obtaining the reciprocal cross, V. rotundifolia x V. cordifolia.

Vitis Cinerea × Vitis Rotundifolia

In 1917 fourteen flower clusters on a native imperfect hermaphroditic Cinerea vine were bagged and later dusted with pollen from perfect flowered Rotundifolia vines. No fruits were produced in any of these bags. In 1918 three flower clusters on another imperfect hermaphroditic Cinerea vine were bagged and later dusted with Rotundifolia pollen. No fruits were produced in these bags.

Vitis Rotundifolia × Vitis Cinerea

In 1916 six flower clusters on P-48 were bagged and later dusted with fresh pollen from a staminate Cinerea vine. No fruits developed in any of these bags. In 1917 two flower clusters on N-28, one on E-19, one on James, five on X-32 and seven on Scuppernong vines were bagged and dusted with fresh pollen from a native staminate Cinerea vine. No fruits developed in any of these bags. In 1918 ten flower clusters on the James variety were bagged and later dusted with pollen from a native staminate Cinerea vine. From these bags one normal berry was taken in the fall. This berry contained three apparently normal seeds. These will be planted and the resultant seedlings will be watched with interest.*

Rotundifolia-Munsoniana Hybrid × Vitis Cinerea

In 1917 eight flower clusters on the variety Eden were bagged and later dusted with pollen from a staminate Cinerea vine. No fruit resulted from this work.

Winchell-Rotundifolia Hybrid × Vitis Rotundifolia

In 1917 two flower clusters on the Winchell-Rotundifolia hybrid of 1912 were bagged and dusted with Rotundifolia pollen and one berry resulted. From this berry one seed was obtained. This seed was planted and a seedling resulted which will be more fully described in our next bulletin. In 1918 five flower clusters on the same vine were again bagged and dusted with pollen from Rotundifolia vines. Three berries set. Two of these berries were lost through the hands of irresponsible persons. From the third berry one seed was obtained.

Several berries developed on this vine during this season as a result of the activities of natural agents of cross-pollination. Because this vine is completely self-sterile and is entirely surrounded by thousands of Rotundifolia seedlings and will cross with V. rotundifolia, and as the blooming period of this hybrid and that of the Rotundifolia vines practically coincide, we assume that these berries, which developed under no human control, are the natural results of cross-pollination with Vitis rotundifolia. Three fruits of this type have been produced from which three seeds have been obtained.

Vitis Rotundifolia × Herbemont-Rotundifolia Hybrid

In 1916 two flower clusters on a seedling vine of Hope parentage were bagged and dusted with pollen from the Herbemont-Rotundifolia hybrid of 1913. No fruit set from this pollination. In 1917 eight flower clusters on Scuppernong vines, one on N-28, one on O-23 and three on Eden were bagged and dusted with pollen from this Herbemont-Rotundifolia vine. One fruit set on the Scuppernong vine, which yielded one seed. No fruits were obtained from any of the other cross-pollinations. Although very little fruit set, we have learned that Vitis rotundifolia will occasionally cross with its F_1 generation with V. bourquiniana.

Vitis Rotundifolia × Vinifera-Rotundifolia Hybrid

In 1918 four flower clusters on K-19 were bagged and dusted with pollen from the Vinifera-Rotundifolia hybrids of 1917 origin. Two berries resulted from this cross, from which two seeds were obtained. Here again we have evidence of Vitis rotundifolia hybridizing with its F_1 generation hybrid vines.

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* Since the writing of the manuscript for this bulletin one of these three seeds has germinated. This seedling from all appearances is a true Rotundifolia and therefore not of hybrid origin.

Parthenocissus Quinquefolia (Planch.) × Vitis Rotundifolia

In 1916 eleven flower clusters on a native vine of Parthenocissus quinquefolia (Planch.) were bagged and the flowers, 410 in number, were carefully emasculated and later, when the pistils matured, pollen from some Rotundifolia vine was applied. No fruits developed in any of these bags.

Vitis Rotundifolia × Parthenocissus Quinquefolia (Planch.)

In 1916 nine flower clusters on a Flowers vine were bagged and dusted with fresh pollen from P. quinquefolia. No fruits developed in any of these bags. Four flower clusters were also bagged on Munson's Sanmonta vine which in all probability is a Rotundifolia-Munsoniana hybrid. The flowers were dusted with fresh pollen from P. quinquefolia. No fruits developed in any of these bags. Our efforts so far have been fruitless with both crosses.

Parthenocissus Tricuspidata (Planch.) × Vitis Rotundifolia

In 1916 ten flower clusters (460 flowers) on a local vine of Parthenocissus tricuspidata were carefully emasculated and as the pistils matured they were dusted with pollen from a Rotundifolia vine. None of these flowers produced a berry.

Vitis Rotundifolia × Parthenocissus Tricuspidata (Planch.)

In 1916 six flower clusters on the variety Flowers were bagged and dusted with fresh pollen from P. tricuspidata. No fruits developed in these bags.

Five flower clusters on the variety Sanmonta were bagged and dusted with pollen from P. tricuspidata. No fruits developed from any of these flowers.

Our effort so far to hybridize Parthenocissus tricuspidata with V. rotundifolia and vice versa has proven fruitless.

Ampelopsis Heterophylla (Sieb. and Zucc.) Variety Elegans (Koch) × Vitis Rotundifolia

In 1918 four flower clusters on a vine of Ampelopsis heterophylla (Sieb. and Zucc.) variety Elegans (Koch) were carefully emasculated and dusted with pollen from V. rotundifolia. No fruits developed from any of these flowers.

4. OBSERVATIONS AND DISCUSSIONS.

While attempting the work of finding limits in the hybridization of Vitis rotundifolia with related species and genera, the following observations and deductions were made.

a. In order to adhere strictly to the letter and object that is before us, only pure species so far as possible were used. Whenever varieties of Euvitis are mentioned in connection with species, Vinifera excepted because these constitute the species, the evidence thus secured should be looked upon as pertaining to the variety only. Thus the varieties Concord and Lutie have been utilized in hybridization work with V. labrusca. Now it is generally recognized that nobody can say with absolute certainty that these varieties are pure Labrusca and that no Vinifera blood is in any way mixed up with them.

Again, as V. rotundifolia will hybridize more readily with some species than with others, so these varieties, if tainted with blood of another species may be either positively or negatively affected in regard to their hybridizing qualities. For example, V. vinifera vines will readily hybridize when V. rotundifolia pollen is used while V. labrusca vines cannot as readily be hybridized. Now Winchell, a Vinifera-Labrusca-Æstivalis hybrid, is readily hybridized with V. rotundifolia pollen; and is it not possibly because of the presence in the hybrid of Vinifera blood?

b. In hybridizing perfect flowered varieties when the species are divergent in character, it has been found advisable for the sake of economy in time and labor not to lay too much stress on the absolute eradication of pollen from the emasculated flowers at the expense of possibly injuring their delicate pistils. We have discovered and possess evidence which shows that Rotundifolia hybrids are not composed of all dominant characters as has long been supposed, but that they are more of an intermediate type and an experienced eye can readily pick them out from a mixed lot of vines.

c. When hybridizing different species it is advisable to have at hand the freshest pollen obtainable. This may be accomplished in several different ways, chief among which are the following: (1) by forcing late blooming vines in an artificially heated glass house, (2) by retarding the blooming period of early flowering vines, (3) by diligent search for the latest flower clusters on early blooming vines, (4) by forcing, if possible, a second crop of flower clusters on a vine, (5) by collecting and storing the pollen from early blooming vines for later use.

d. Some varieties are frequently found that will not readily yield positive results in hybridization, while others of the same species may be found that will. For example, the variety Scuppernong with us has not yielded to the pollen of V. vinifera while our Oberlin vine has produced three berries. Deering (5) mentions a similar experience.

We (6) have found a vine of our own production which, according to our knowledge has not yet been fertilized with pollen from other vines of its own species, but it never fails to set fruit with its own pollen. Very often when it seems impossible to secure a hybrid, when all conditions seem to be complied with, the reverse cross might yield abundant results. As an example of this kind we may cite such as V. vinifera, V. bourquiniana, V. labrusca, V. cordifolia and probably others which readily hybridize with V. rotundifolia and V. munsoniana when the latter two species are used as the pollen parents, but will not hybridize as readily when the positions of the parents are reversed.

e. The hybridizer must not be easily discouraged when positive results are not immediately forthcoming. He must try and try again until success follows his efforts. In the case of the Rotundifolia-Vinifera hybrid, when Vinifera was used as the male parent, we worked with 28 flower clusters which comprised no less than 500 individual flowers before a hybrid was secured.

f. When hybrid seeds have finally been secured, great care must be exercised in the handling of the seedbed, otherwise untoward conditions may kill the hybrid vines and nullify all the previous work. The damping-off disease is the archenemy of the seedlings in the seedbed, and to protect the young plants from it requires the greatest of care and vigilance. With the amateur, the word hybrid is very often considered as almost synonymous with great vigor and strength, and the fact that hybrids of wide parentage ofter require the greatest of care and protection is learned only when too late.

g. Occasionally when we sow seeds of supposedly hybrid origin and when the utmost care has been bestowed on the emasculation of hermaphroditic grape flowers, we get a mixed lot of selfed seedlings, together with hybrids, if by chance such were produced. Beach (1) mentions this self-fertilization of the grape flowers in the bud in the case of the variety Mills, Dorsey (2) in the case of the varieties Concord and Hubbard Seedless, Hedrick and Anthony (3) mention other varieties of Euvitis behaving similarly. Our self-fertile varieties and seedlings of V. rotundifolia have proven to be no exception to this fertilization in the bud. Euvitis varieties with us have likewise given similar results. If this phenomenon is not carefully considered, we may be led into erroneous deductions in regard to the transmission of characters from the parental to the F_1 generation vines. When two varieties of the same species are to be hybridized, the selfed seedlings may not be detected and contradictory results may develop; if, however, the parental vines are of widely different types, the two kinds of offspring seedling vines can generally be recognized at once by their general appearance. It is here that several prominent Rotundifolia grape breeders apparently have fallen into erroneous conclusions.*

h. Finally the hybrid nature of a seedling is proof positive that hybridization has been effected between two given individuals.

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* See False Hybrids in our next bulletin which will be entitled, "Some F Hybrids between Vitis Rotundifolia and Related Species."

SUMMARY

As a result of our efforts to determine the limits in hybridization of Vitis rotundifolia with related species and genera, we have come to conclusions which may be summarized as follows:

1. Vitis rotundifolia will hybridize with V. munsoniana and some species of Euvitis, namely: V. vinifera, V. bourquiniana, V. labrusca, V. cordifolia, and V. æstivalis, also with the varieties Winchell, Concord, and others. Other species will be reported on later as time and material will permit.
2. It is doubtful if V. rotundifolia will hybridize with all species of Vitis.
3. Our efforts so far indicate that V. rotundifolia will not hybridize either way with Parthenocissus quinquefolia (Planch.), P. tricuspidata (Planch.) or Ampelopsis heterophylla (Sieb. and Zucc.) var. Elegans (Koch).
4. Vitis rotundifolia will hybridize with its own F_1 hybrids with other species of Vitis.
5. Vitis rotundifolia when used as the male parent will hybridize quite readily with some species of Euvitis, but when used as the female parent it will hybridize only rarely. Some combinations seem more congenial for hybridization than do others.
6. The results that are reported in this bulletin do not establish any limit, but will indicate what may be expected in the hybridization of V. rotundifolia with the mentioned related species and genera.

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AUGUST 1919 TECHNICAL BULLETIN No. 18

Some F_1 Hybrids of Vitis Rotundifolia With Related Species and Genera

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TABLE OF CONTENTS

PAGE
I. List of Illustrations ---- 6
II. Introduction ---- 9
III. Hybrids represented in this discussion ---- 9
IV. Description of hybrids ---- 11
V. A general survey of the characters in the F_1 and subsequent generations ---- 37
VI. So-called "false hybrids" ---- 39
VII. Summary ---- 47
VIII. Literature cited ---- 51

LIST OF ILLUSTRATIONS

Fig. 1. Winchell-Rotundifolia hybrid vines of 1913 in foliage. This photograph shows the tardy growth that is characteristic of this class of hybrids. Photographed May 16, 1919. Reduced.

Fig. 2. A Winchell-Rotundifolia hybrid vine. This vine was grown from seed in the spring of 1913 and is the oldest living known hybrid between Euvitis and V. rotundifolia. Unpruned in the dormant stage, winter of 1918-1919. Reduced.

Fig. 3. Sections of two canes from the 1913 Winchell-Rotundifolia hybrid vine showing practically continuous pith, which is characteristic of the Rotundifolia parent species. Reduced.

Fig. 4. Sections of canes from each of the two parents and of the hybrid vines. The cane at the left is V. rotundifolia (light male), the one in the center is Winchell (female parent), the one on the right is the hybrid.

The Winchell cane shows the well defined diaphragms that interrupt the pith column at every node while the other two canes show no such diaphragms. The hybrid cane, furthermore, exemplifies its hybrid nature in its relatively less zigzag growth, the slightly enlarged nodes, the internodes, which are intermediate in length, and the pith column whose relative diameter is intermediate between that of both its parents. Reduced.

Fig. 5. A leafy shoot from the 1913 Winchell-Rotundifolia hybrid, showing its type of shoot, leaf and tendril. Reduced.

Fig. 6. Three typical leaves representing both parents and hybrid. Upper leaf is from Winchell (female), lower right is from Rotundifolia (male) and lower left is from the hybrid. The size of the hybrid leaf is largely determined by the Rotundifolia parent while the character of the leaf is more intermediate. Reduced.

Fig. 7. Types of flower-clusters represented by Winchell (perfect hermaphroditic but used as the female parent) on the left; hybrid (imperfect hermaphroditic) second; Scuppernong (imperfect hermaphroditic) third; and staminate Rotundifolia (male parent) on the right. The sizes of the flower-clusters in our native species of Vitis are sex-limited; the cluster of this hybrid vine which is an imperfect hermaphrodite, therefore, is smaller than that of either of its parents, it takes after the size of the flower-cluster that is associated with the imperfect hermaphroditic Rotundifolia vine and, therefore, is determined by the Rotundifolia species. Reduced.

Fig. 8. Seeds from parental and hybrid vines; on the left are seeds from Winchell (female parent), on the right are typical Rotundifolia seeds (representing the male parent), below are seeds from the hybrid vine. The general outline and type of the seeds from the hybrid vine evidently are determined by the Rotundifolia parent. Natural size.

Fig. 9. Two types of flower-clusters found among the F_1 hybrid vines when Winchell is crossed with the perfect hermaphroditic Rotundifolia vine I-1. The cluster on the left (perfect hermaphroditic) assumes the size of the perfect hermaphroditic Rotundifolia vine, while the cluster on the right (imperfect hermaphroditic) assumes the size of similar flower-clusters that are found on Rotundifolia vines. Reduced.

Fig. 10. A sample of the pollen that is produced by the perfect hermaphroditic hybrid vines of Winchell-Rotundifolia parentage. Notice the three

apparently normal pollen grains marked with an arrow and found in the upper right-hand corner. Magnified 230 diameters.

Fig. 11. A leafy shoot with a flower-cluster from the 1914 staminate Bourquiniana-Rotundifolia hybrid (Herbemont × Light Male No. 2). This is the oldest known Bourquiniana-Rotundifolia hybrid vine. Notice the five-lobed leaves which are characteristic of these hybrids; the short, simple and bifid tendrils, the small flower-cluster (similar to an average staminate Rotundifolia flower-cluster) and the slender and nearly straight cane. Reduced.

Fig. 12. Three typical leaves representing both parent and hybrid. Upper leaf is from Herbemont (female parent), lower left is from Rotundifolia (male parent), lower right is from the hybrid. The size of the leaf on this hybrid is determined by the Rotundifolia parent, but the lobing is much more pronounced than in the mature stage of the Herbemont parent, however, the juvenile leaves of Herbemont are deeply lobed. Reduced.

Fig. 13. A flower-cluster of each of the parents and of the hybrid vine. The large flower-cluster at the right is from Herbemont (perfect hermaphroditic but used as the female parent), upper cluster at the left is from staminate Rotundifolia vine (male parent), lower cluster is from the 1913 hybrid vine (staminate). The size of the flower-cluster on this hybrid is determined by the Rotundifolia species. Reduced.

Fig. 14. A Vinifera-Rotundifolia hybrid vine (Malaga Seedling No. 1 × G-52) showing the character of growth, shoots, leaves, tendrils, etc. of this vine. Not all hybrids of this lot resemble the vinifera parent as closely as does this vine. Reduced.

Fig. 15. A leafy shoot with flower-clusters from a Vinifera-Rotundifolia hybrid vine, showing the comparative sizes of the flower-cluster, leaf and tendril; also a different type of lobing of the leaves from that of Fig. 14. Reduced.

Fig. 16. Leaves from V. vinifera, V. rotundifolia, and from a hybrid vine. Upper leaf is from Malaga Seedling No. 1 (female parent), lower right-hand leaf is from a Rotundifolia vine (male parent), lower left-hand leaf is from a hybrid vine. The size of the hybrid leaf is largely determined by the Rotundifolia species while the character and the lobing seem to be more or less intermediate between that of both the parents. Reduced.

Fig. 17. A sample of leaves from the Vinifera-Rotundifolia hybrid vines, showing the differences that can be observed in the lobing of the leaves. Some are almost like Vinifera while others are almost like Rotundifolia leaves. Reduced.

Fig. 18. Typical flower-clusters of V. vinifera, V. rotundifolia and of a hybrid vine. The cluster at the left is from Malaga Seedling No. 1, imperfect hermaphroditic (female parent); the one at the right is from G-52, perfect hermaphroditic (male parent); the one in the center is from a hybrid vine, perfect hermaphroditic. The small size of the flower-clusters of this and similar hybrid vines is determined largely by the Rotundifolia species. Reduced.

Fig. 19. Two types of flower-clusters found among the F_1 hybrid vines when Malaga Seedling No. 1 (imperfect hermaphroditic) is crossed with G-52 (perfect hermaphroditic). The cluster on the left (perfect hermaphroditic) and the cluster on the right (imperfect hermaphroditic) are typical in size of similar flower-clusters found among vines of the Rotundifolia species, otherwise the clusters are hybrid in character. Reduced.

Fig. 20. A sample of the pollen that is produced by the perfect hermaphroditic hybrid vines of Vinifera-Rotundifolia parentage. Notice the comparatively few, about 14.4 per cent, normal pollen grains marked with an arrow. Magnified 230 diameters.

Fig. 21. A leaf and a fruit-cluster, from a Vinifera-Rotundifolia hybrid vine (Malaga Seedling No. 1 × G-52). Notice the small fruit-cluster which is characteristic of the Rotundifolia species. Natural size.

Fig. 22. Seeds from the parent vines and from one of the hybrid vines. Malaga Seedling No. 1 (female), G-52 (male). The general outline and appearance of these hybrid seeds are largely determined by the Rotundifolia species. Natural size.

Fig. 23. A leafy shoot from a Rotundifolia-Vinifera hybrid vine (Oberlin vine as female and Malaga as male). Neither of the parent vines has a decidedly lobed leaf. Reduced.

Fig. 24. A hybrid seedling (in a three-inch flower pot) of Oberlin vine when crossed with pollen from Malaga. The leaves of this vine are more like Rotundifolia than are those of the preceding. This vine is beginning the second year of its existence. Reduced.

Fig. 25. An F_1 hybrid between V. cordifolia (female) and V. rotundifolia (male), 1918. Two existing vines of this type are the first hybrids to be recorded between these two species of grapes. The character of the growth is very much that of the female parent species. Reduced.

Fig. 26. An F_1 hybrid vine between V. labrusca, var. Concord (female) and V. rotundifolia, G-52 (male). The vine is in a three-inch flower pot and going on to its second year of growth. Notice the two simple tendrils. A bifid tendril is on the other side of the vine but not visible. Reduced.

Fig. 27. An F_1 hybrid vine between V. aestivalis (female) and V. rotundifolia, G-52 (male). This vine has been grown from seed that germinated in March of 1919. The Rotundifolia leaf characters are plainly visible in this vine. Reduced.

Fig. 28. Leafy shoots from six of Munson's so-called "false hybrids." Reading from left to right they are: (1) Sanalba, (2) Labama, (3) LaSalle, (4) San Jacinto, (5) San Melaska, (6) Sanmonta. Notice that all the characters of the tendrils, shoots and leaves are those of true Rotundifolia vines. Reduced.

Fig. 29. Selected shoots from a Rotundifolia vine, variety Scuppernong. Notice that many of the tendrils are bifid. Reduced.

Fig. 30. Selected shoots from Rotundifolia seedling vines, showing variations. Leaves of upper shoots show a decided tendency toward lobing while the tendrils of the lower shoots show that all tendrils on Rotundifolia vines are not simple. Reduced.

Fig. 31. Selected shoots of a Rotundifolia vine showing that even trifid tendrils may be found within this species. This trifid tendril was first found on a wild (Oberlin) vine and later has been transmitted to a number of its progeny. Reduced.

Fig. 32. A photograph of a branch of a Luola vine (V. rotundifolia), showing that fairly large clusters of fruit may be produced on Rotundifolia vines. Reduced.

Fig. 33. Two clusters of fruit from a Rotundifolia (Oberlin) vine which shows that all Rotundifolia fruit-clusters are not small. Natural size.

SOME F_1 HYBRIDS BETWEEN VITIS ROTUNDIFOLIA AND RELATED SPECIES*

L. R. DETJEN, *Assistant Horticulturist*

INTRODUCTION

In the search for information regarding the limits of hybridization of Vitis rotundifolia, numerous hybrid plants of different parentage have been produced. Up to the present time only nine different combinations of parents are represented, but from even this small number much valuable information has been obtained as to the nature of the characters found in the F_1 hybrid vines.

Since the hybrid character of the seedling is proof positive that hybridization has been effected between two given individuals, we publish herewith careful descriptions of some of these hybrid vines that the evidence may be conclusive. Furthermore, an analysis of these hybrids has led us to make comparisons of these with certain well-known and so-called "false hybrids" and other vines that are claimed to be hybrids between Muscadinia and Euvitis, and to point out wherein these are not hybrids as represented, but only pure seedlings of the Muscadine group.

The following list of combinations of species and varieties includes representatives of all the hybrid vines that we have so far produced and are herein described, but only seedling vines from the first three parental combinations are old enough to permit of a thorough and minute description.

A. LIST OF PARENTAL COMBINATIONS EFFECTED

I. Winchell (Vinifera-Labrusca-Aestivalis) × Rotundifolia 1912 and 1916.
II. Herbemont (Bourquiniana) × Rotundifola 1913, 1916 and 1917.
III. Malaga Seedling No. 1 (Vinifera) × Rotundifolia 1916.
IV. Malaga Seedling No. 3 (Vinifera) × Rotundifolia 1917.
V. Malaga (Vinifera) × Rotundifolia 1917.
VI. Oberlin Vine (Rotundifolia) × Malaga (Vinifera) 1917.
VII. Vitis cordifolia (native species) × Rotundifolia 1917.
VIII. Concord (Labrusca) × Rotundifolia 1917.
IX. Winchell-Rotundifolia hybrid × Rotundifolia 1917.

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* Original manuscript submitted January, 1919.

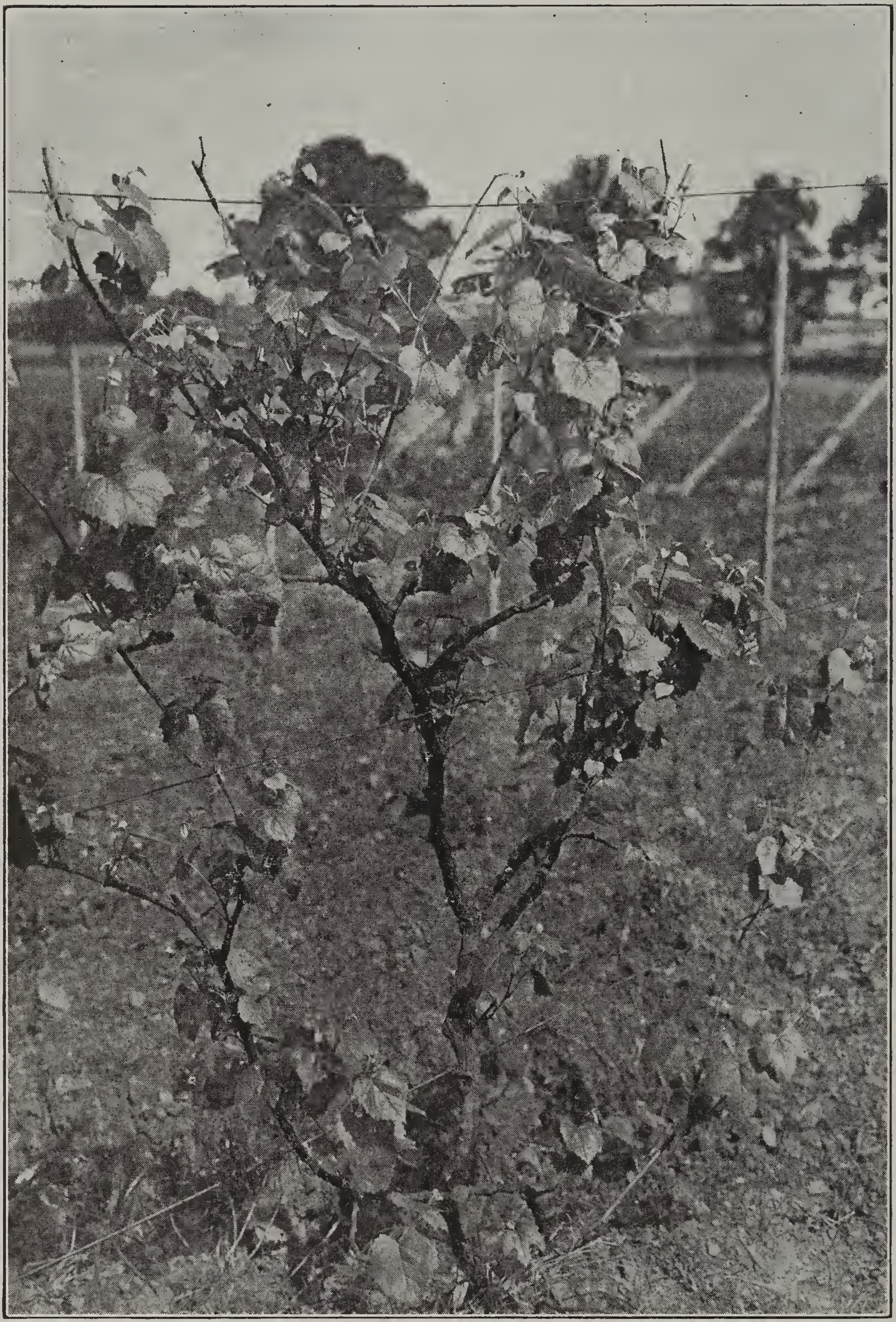

Fig. 1. Winchell-Rotundifolia hybrid vine of 1913 in foliage. This photograph shows the tardy growth that is characteristic of this class of hybrids. Photographed May 16, 1919. Reduced.

B. DESCRIPTION OF THE CHARACTERS OF HYBRID VINES

I-a. Winchell × Rotundifolia (Light Male), 1912.

General character of plant: During the first few years the hybrid plant seemed weak in growth, in this respect greatly resembling the

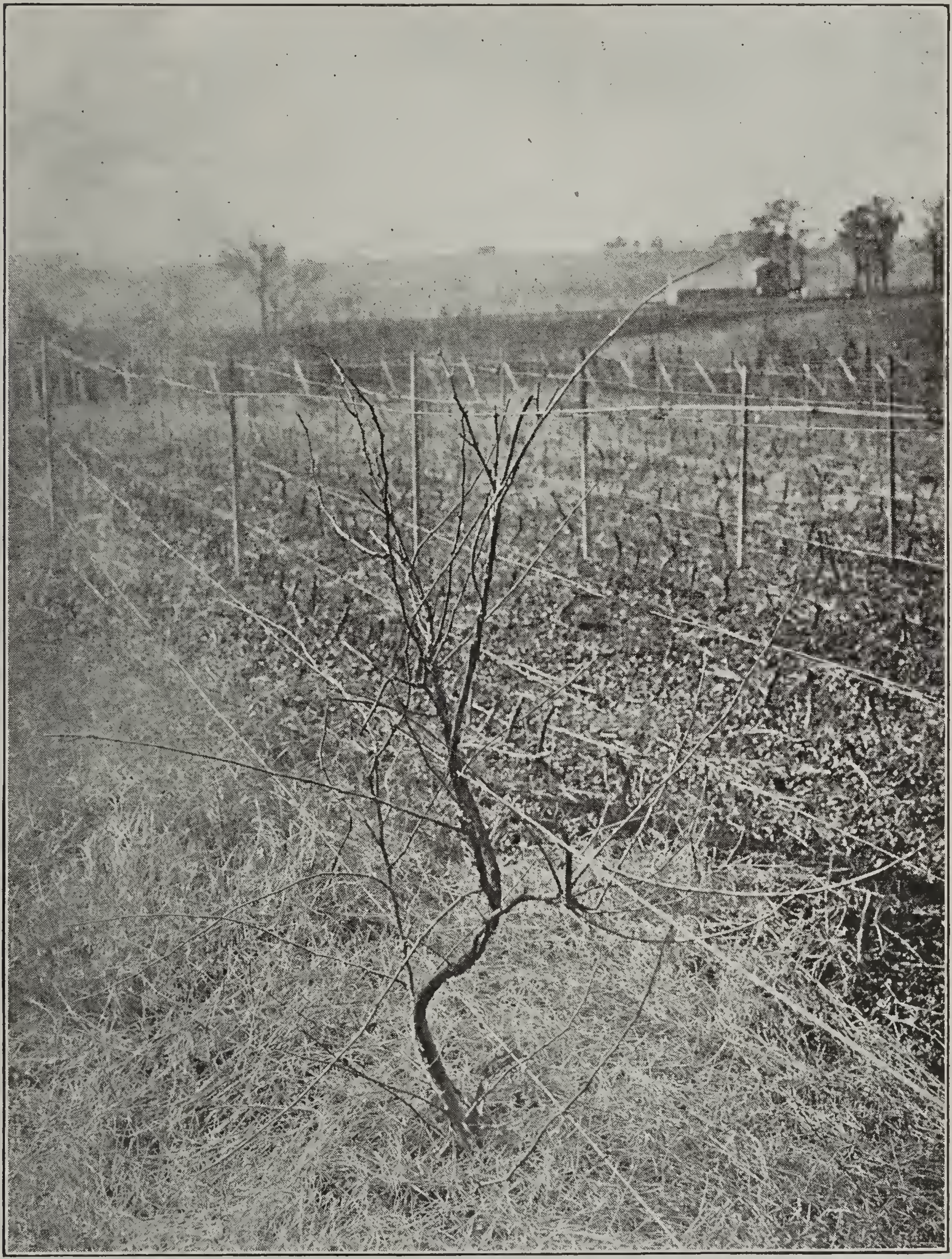

Fig. 2. A Winchell-Rotundifolia hybrid vine. This vine was grown from seed in the spring of 1913 and is the oldest living known hybrid between Euvitis and V. rotundifolia. Unpruned in the dormant stage, winter of 1918-1919. Reduced.

selfed seedlings of Winchell. The plant is not inclined to be rampant in growth, but instead is low and spreading. In general appearance the hybrid vine may be said to resemble the Winchell vine more than the Rotundifolia parent, but a critical examination of individual characters reveals the true hybrid nature of the vine.

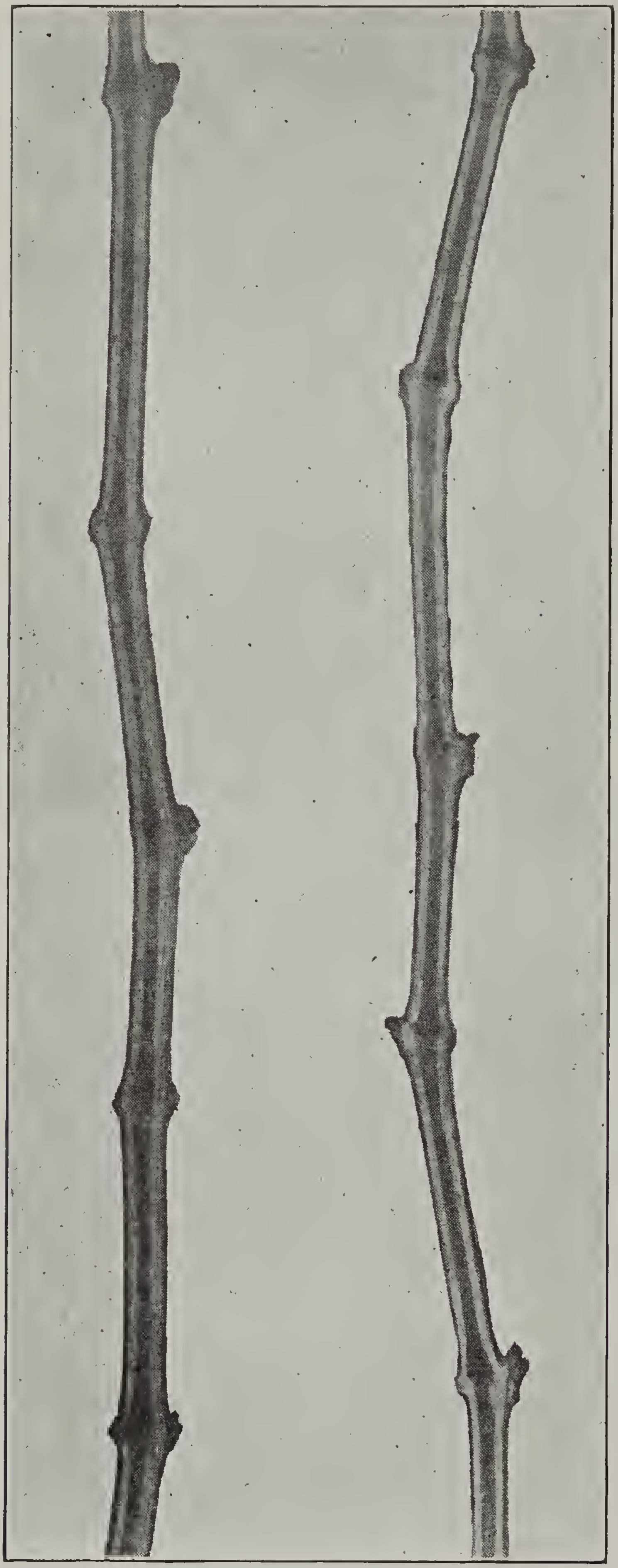

Fig. 3. Sections of two canes from the 1913 Winchell-Rotundifolia hybrid vine showing practically continuous pith which is characteristic of the Rotundifolia parent species. Reduced.

Vigor of vine: Fairly strong but not vigorous, not hardy, very slow to leaf out in the spring, canes die back perceptibly every winter.

Canes: Fairly strong but neither stout nor slender, nearly straight, not bent at the nodes, slightly compressed, angled especially when young. Sometimes at the base the canes become round and smooth. The striation is very obscure.

Wood: Soft, one-year-old wood is greenish-yellow in color; easily grafted onto roots of Euvitis.

Pith: Green and occasionally brown in color, full of sap. The green pith during the dormant season is crowded full of starch grains. The brown pith is composed of dead cells and when dry sometimes splits into discs. Very little, if any, starch grains remain in this pith during the dormant season. Sometimes cells of this brown pith are found scattered among the green.

The pith in general may be said to be continuous through the nodes. It is only very seldom that it is inter-

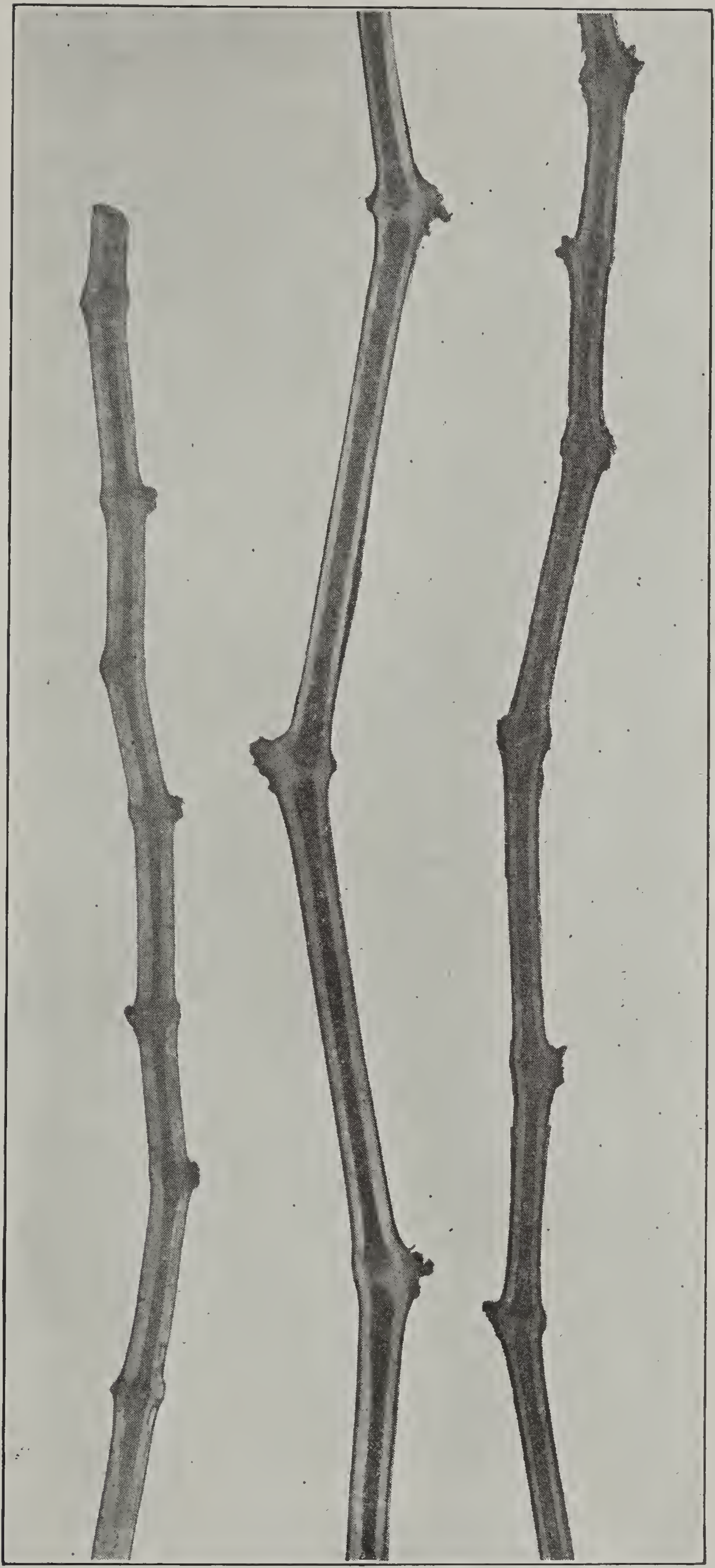

Fig. 4. Sections of canes each of the two parents and of the hybrid vine. The cane at the left is V. rotundifolia (light male), the one in the center is Winchell (female parent), the one on the right is the hybrid.

The Winchell cane shows the well defined diaphragms that interrupt the pith column at every node while the other two canes show no such diaphragms. The hybrid cane, furthermore, exemplifies its hybrid nature in its relatively less zigzag growth, the slightly enlarged nodes, the internodes, which are intermediate in length, and the pith column whose relative diameter is intermediate between that of both its parents. Reduced.

rupted by well defined diaphragms. With some exceptions the tissue at the nodes is composed of true pith cells but these are often so compressed as to give the whole structure the appearance of a partition as seen with the unaided eye. Sometimes, however, we do find true hard and woody diaphragms. Often we find a complete separation or rent in the pith column just above the place where a diaphragm is supposed to be. The average diameter of the pith column of the hybrid is a little less than that of the two parent vines.

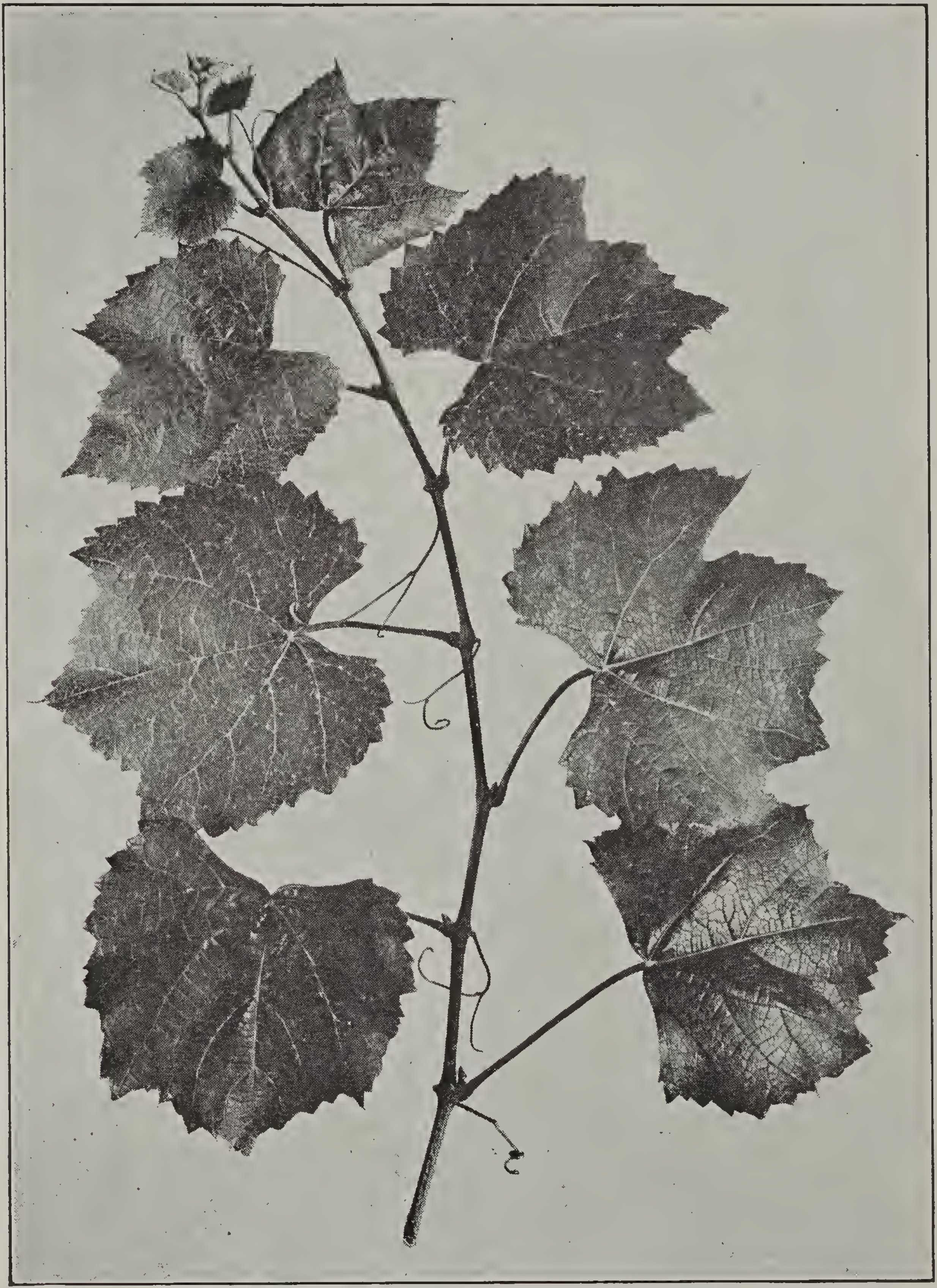

Fig. 5. A leafy shoot from the 1913 Winchell-Rotundifolia hybrid, showing its type of shoot, leaf and tendril. Reduced.

Bark: Reddish brown, covered by a thin layer of gray, rough; lenticels inconspicuous, submerged; bark persistent on young wood and shedding from old wood in broad plates as in Muscadinia.

Nodes: Somewhat enlarged on bud side.

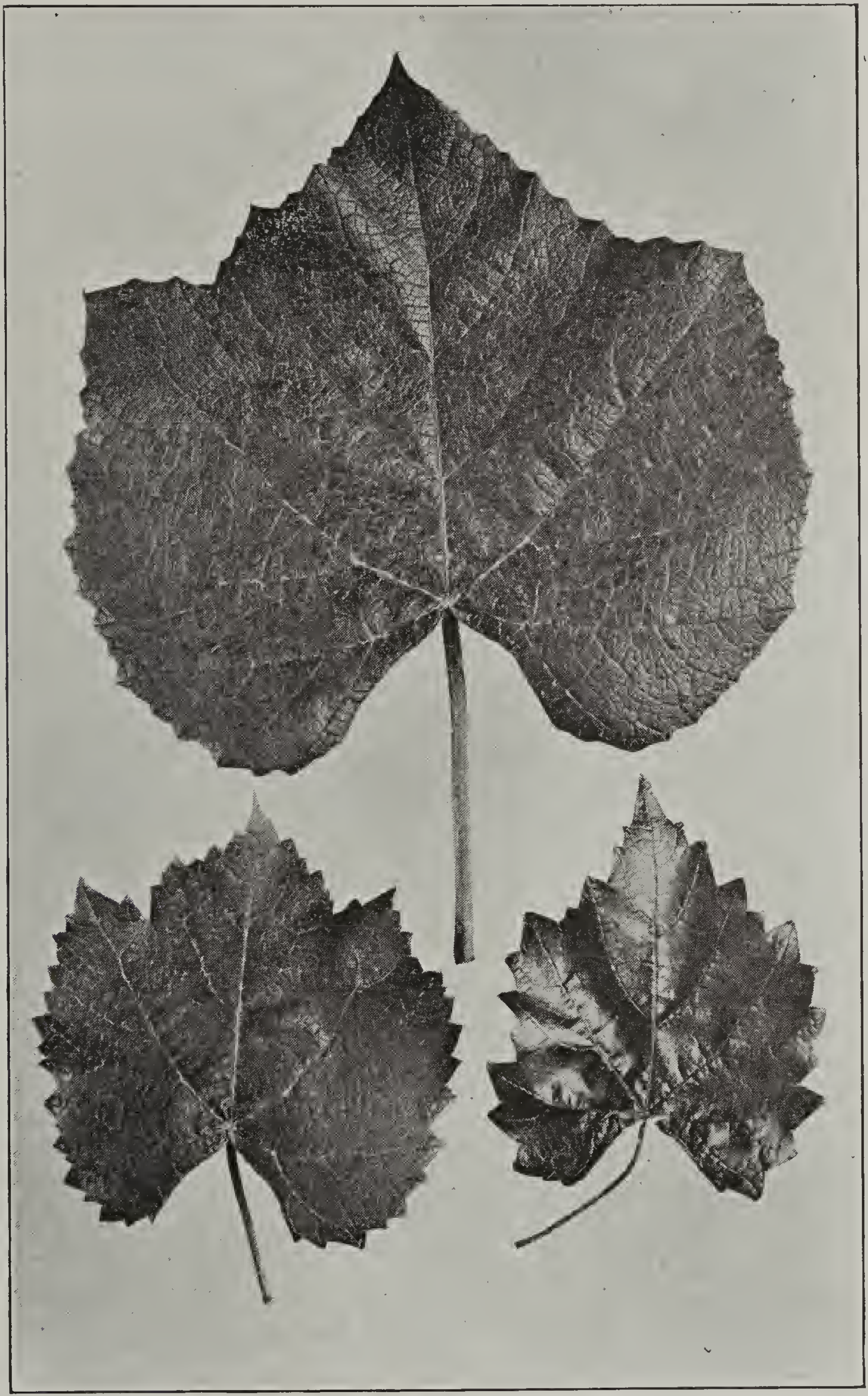

Fig. 6. Three typical leaves representing both parents and hybrid. Upper leaf is from Winchell (female), lower right is from Rotundifolia (male) and lower left is from the hybrid. The size of the hybrid leaf is largely determined by the Rotundifolia parent while the character of the leaf is more intermediate. Reduced.

Internodes: Much shorter than in the variety Winchell, but somewhat longer than in Rotundifolia.

Leaf buds: Small, conical in shape, similar in this respect to those of Winchell, but in size like Rotundifolia, dark brown in color. Young leaf buds often greatly resemble true Rotundifolia buds. Basal leaf buds on new canes usually unfold the same season much like Winchell or V. vinifera.

Shoots: Very much like those of Winchell, leaves unfolding rapidly. The growing tips are not naked as in Rotundifolia.

Color of foliage: Leaves, tendrils and young stems are usually light green in color. Sometimes a faint tinge of brown appears on the tendrils and on the nodes. Young unfolding leaves are pale yellowish-green in color, due mainly to the pubescence which envelops the growing tips.

Leaves: A trifle larger than V. rotundifolia with the shape of Winchell; teeth not as prominent as in Rotundifolia but more so than

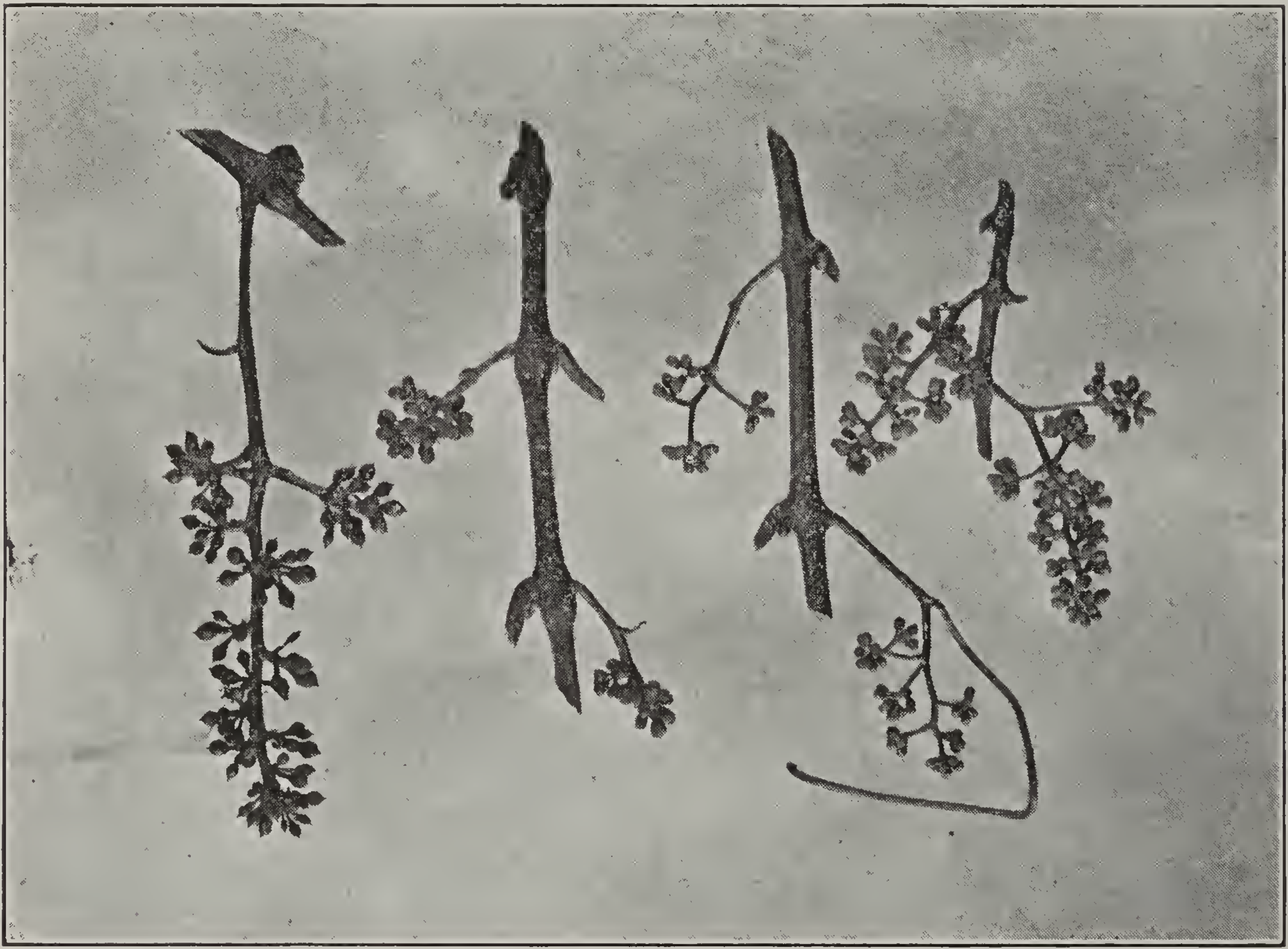

Fig. 7. Types of flower-clusters represented by Winchell (perfect hermaphroditic but used as the female parent) on the left; hybrid (imperfect hermaphroditic) second; Scuppernong (imperfect hermaphroditic) third; and staminate Rotundifolia (male parent) on the right. The sizes of the flower-clusters in our native species of Vitis are sex-limited; the cluster of this hybrid vine which is an imperfect hermaphrodite, therefore, is smaller than that of either of its parents, it takes after the size of the flower-cluster that is associated with the imperfect hermaphroditic Rotundifolia vine and, therefore, is determined by the Rotundifolia species. Reduced.

in Winchell; blades thin, silky, hairy above and cobwebby below; petioles and young canes also cobwebby, glossy dark green above, paler beneath; texture and surfaces of leaves are more like Winchell than Rotundifolia; stipules larger than in Rotundifolia. Leaves are comparatively free from insects and fungi. The peculiar yellowing of the leaves in late summer and fall on Rotundifolia vines is also characteristic of this hybrid. The blackened areas on the leaves due to Guignardia Bidwellii (V. & R.), are small and typical of those commonly seen on leaves of Rotundifolia. The leaves drop from the vine late in the fall with those of Rotundifolia.

Tendrils: Small and weak, growing stronger where a support has been gained, mostly bifid, sometimes simple, silky pubescent when young, usually green but occasionally very light brown in color.

Flower-clusters: Very small and often smaller than on Scuppernong; rachis soft and buds crowded as on Winchell; buds larger than on Rotundifolia.

Flowers: Imperfect hermaphroditic; large pistils and reflexed stamens.

Blooming period of vine: Just after Winchell and practically simultaneous with Rotundifolia. A second crop of flowers may be forced out by summer pruning.

Fertility of flowers: Pollen shriveled and impotent; ovules mostly sterile, occasionally fertile. This vine is sterile because of the double phenomenon of intersexualism with attendant impotence and hybridization.

Fruit-clusters: Very small. Thus far no clusters have been produced that consisted of more than two berries; peduncle very short.

Berries: Medium in size, 16 mm. long and 14 mm. wide, do not

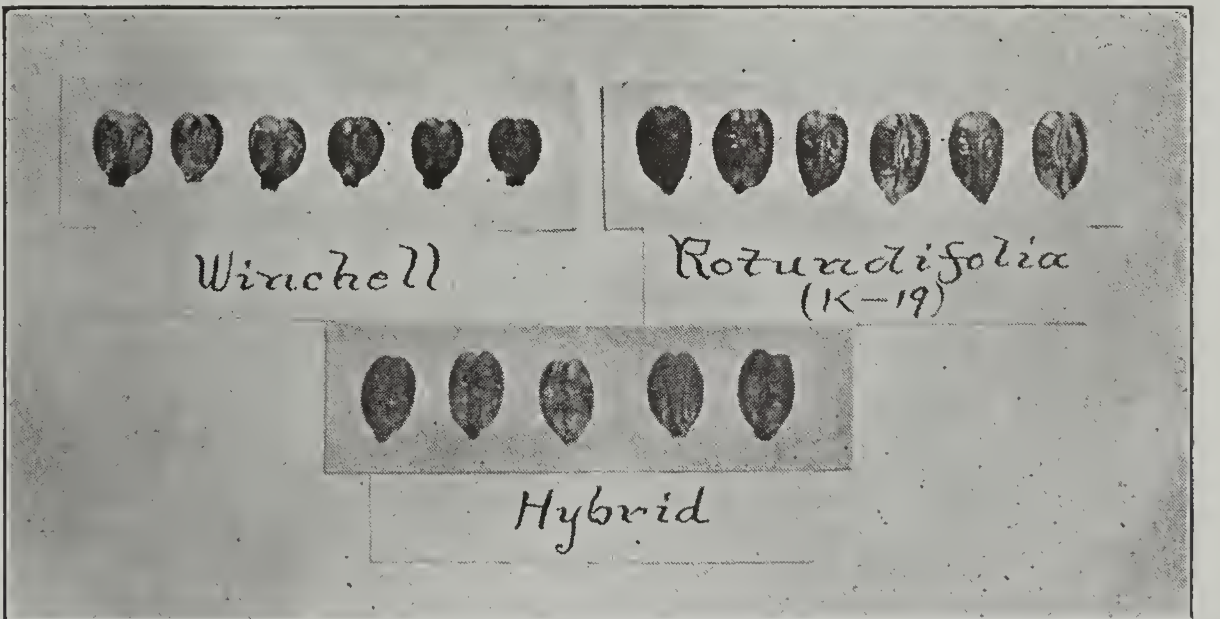

Fig. 8. Seeds from parental and hybrid vines; on the left are seeds from Winchell (female parent), on the right are typical Rotundifolia seeds (representing the male parent), below are seeds from the hybrid vine. The general outline and type of the seeds from the hybrid vine evidently are determined by the Rotundifolia parent. Natural size.

cling well to the stems but do not shatter as readily as the average Rotundifolia; dark red, almost like the variety Emperor in color, decidedly oblong; skin medium thin and tough and covered with a few conspicuous, raised, russet dots; pulp soft, sweet to subacid; very good in quality with an agreeable flavor.

Seeds: Thus far only one seed has been found per berry. The average length of seed (only five seeds having been measured) being 6.3 mm., the average width, 4.0 mm., the average depth, 3.0 mm.; color of the seeds variable ranging from light to dark brown, not glossy; beak the same color as the body of the seed; chalaza located slightly above the middle of the dorsal side of the seed, ovate to oblong in shape, depressed and sometimes sunken; suture broad and deep from the chalaza to the top of the seed and shallow from the chalaza to the beak, slightly wrinkled around the chalaza; raphe hair-like extending from beak to suture on top of the seed where it becomes invisible; ventral depressions deep, broad, curving outward toward the top of the seed,

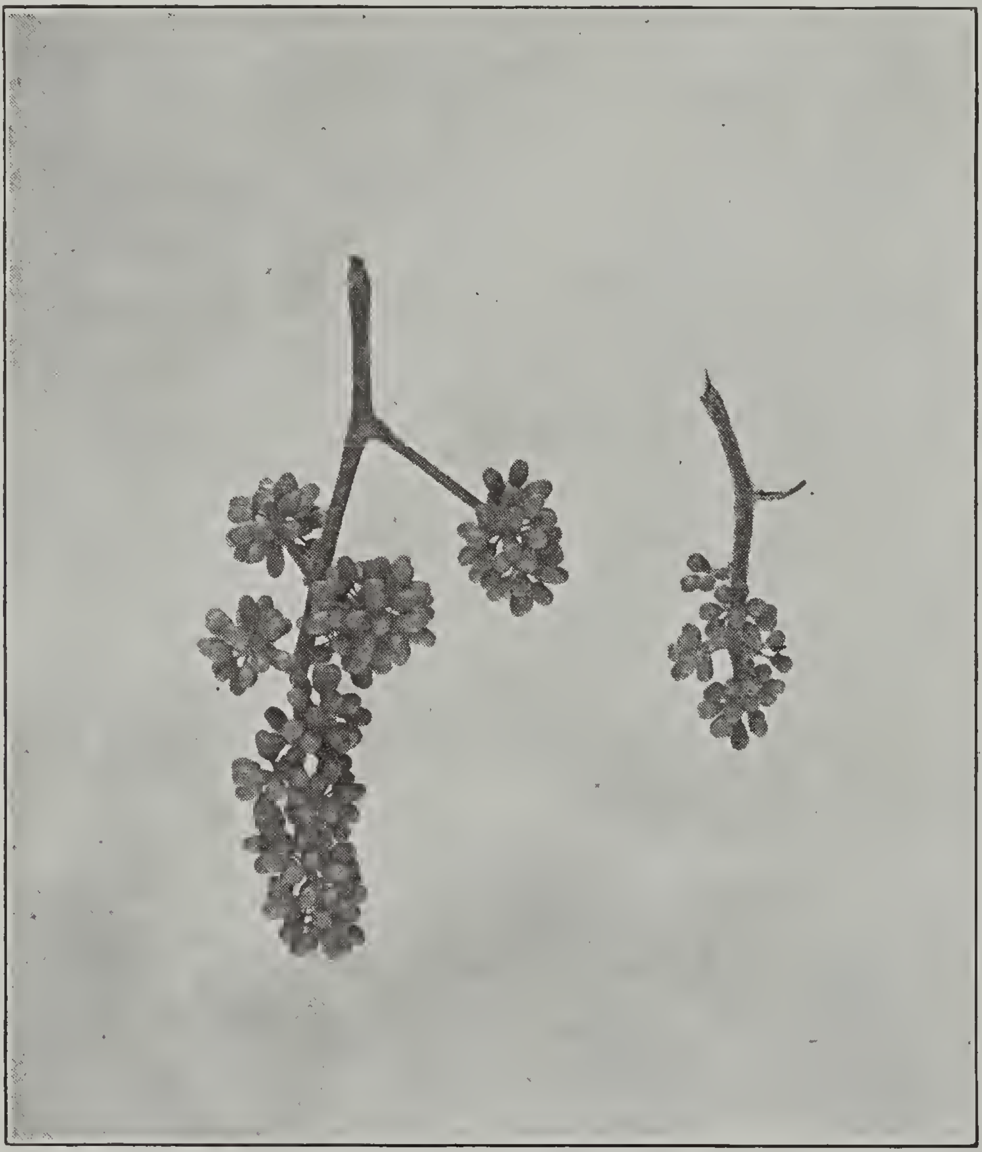

Fig. 9. Two types of flower-clusters found among the F_1 hybrid vines when Winchell is crossed with the perfect hermaphroditic Rotundifolia vine I-1. The cluster on the left (perfect hermaphroditic) assumes the size of the perfect hermaphroditic Rotundifolia vine, while the cluster on the right (imperfect hermaphroditic) assumes the size of similar flower-clusters that are found on Rotundifolia vines. Reduced.

sides of depressions smooth or slightly wrinkled, slightly lighter in color than the body of the seed. The general outline of the seed is oblong with short beak. Its general appearance is that of Rotundifolia, but the broad, deep suture on the top of the seed together with the color and form of the ventral sutures is indicative of the Winchell seed.

I-b. WINCHELL × ROTUNDIFOLIA (I-1 and G-52), 1916.

These plants differ from the one preceding in only a few respects. Since the male parent of these vines is a true hermaphroditic Rotundifolia, two types of hermaphroditic vines have appeared, the perfect and the imperfect.

The perfect hermaphroditic vines bear the larger type of flower-cluster that is found associated with the male and hermaphroditic Rotundifolia vine, but the texture of the cluster tissues and the general appearance of the flower buds is more nearly that of the Winchell parent. The imperfect hermaphroditic vines are similar to the one previously described.

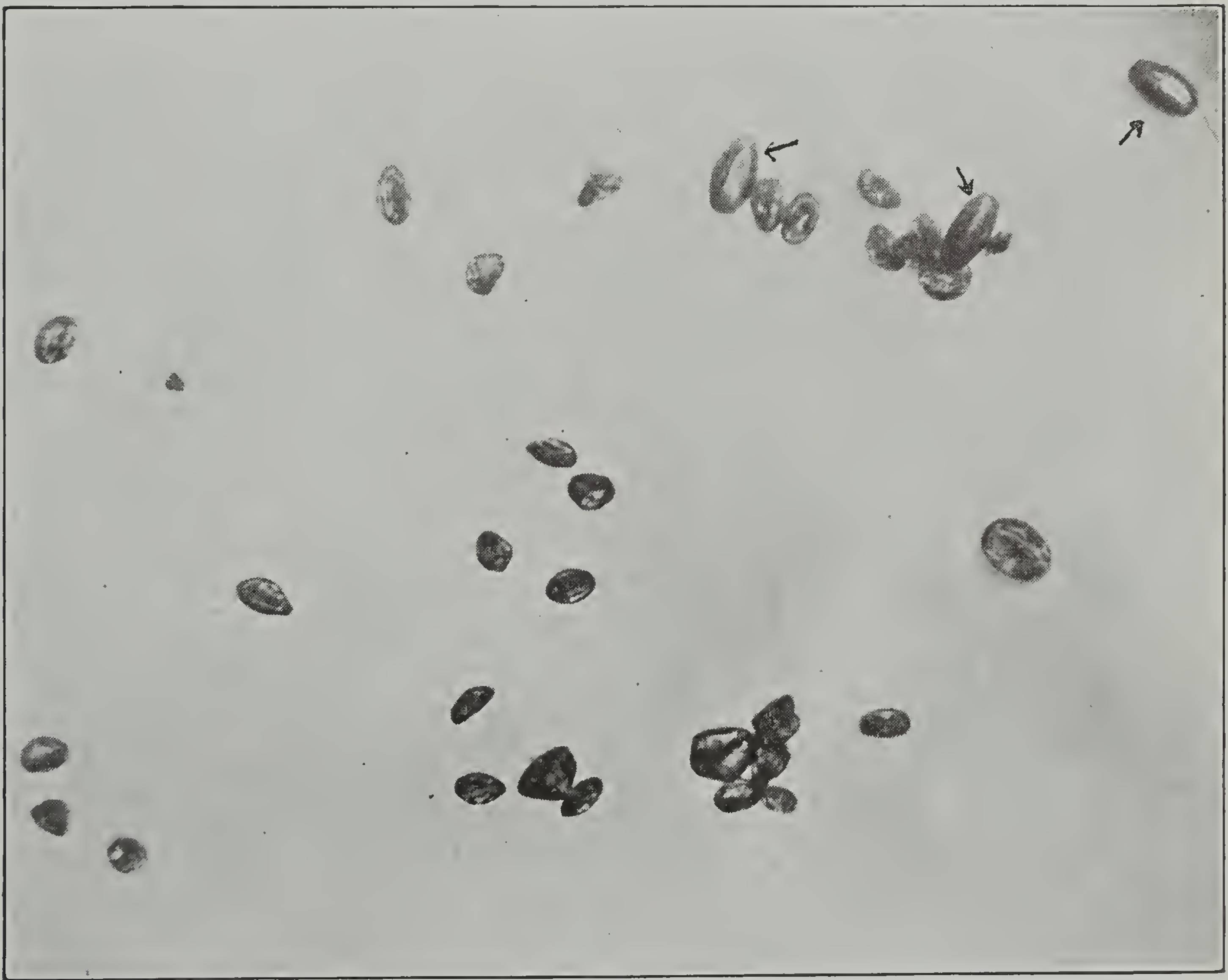

Fig. 10. A sample of the pollen that is produced by the perfect hermaphroditic hybrid vines of Winchell-Rotundifolia parentage. Notice the three apparently normal pollen grains marked with an arrow and found in the upper right-hand corner. Magnified 230 diameters.

The majority of the pollen grains of the perfect-flowered vines are much shriveled, angular and impotent. Some normal and viable pollen grains (about 7.7 per cent) are produced as was discovered by the aid of the microscope and in actual hand pollination experiments. These vines are practically sterile because of hybridization.

While these perfect-flowered hybrid vines did not set fruit in 1918, it is safe to say that some of the pistils in later years will prove fertile. This conclusion is based on work done with the imperfect hermaphroditic Winchell-Rotundifolia hybrid vine of 1912, and the Vinifera-Rotundifolia perfect-flowered hybrid vine of 1916.

The blooming period of these hermaphroditic vines is about midway between that of the two parents.

II-a. HERBEMONT × ROTUNDIFOLIA (Light Male), 1913.

General character of plant: Very weak, not hardy, dies back very badly during the winter if not well protected, very low and bushy. After five years of alternate growing and dying back, the plant measures not more than two feet in height. This plant has neither the appearance of Herbemont nor that of Rotundifolia.

Vigor of vine: Very weak, but more vigorous than some Herbemont seedlings.

Canes: Short and slender, straight, not much bent at the nodes, angled when young, somewhat rounded on older wood, not much compressed, faintly striated.

Wood: Not as hard as that of Rotundifolia, greenish-white in color, easily grafted.

Pith: Greenish-brown to brown, sappy, usually filled with stored starch grains, practically continuous through the nodes, occasionally a woody diaphragm or part of one may be observed. Pith cells at the nodes are compressed and densely packed, thus producing somewhat the appearance of a diaphragm. Column of pith when compared to the diameter of the cane is larger than in Rotundifolia and smaller than in Herbemont.

Bark: Dark reddish-brown overcast with gray, rough; lenticels submerged and inconspicuous; bark persistent in young canes, nonshredding, breaking off in irregular plates and patches when old; no bloom on the bark of the vine.

Nodes: Slightly enlarged on bud side.

Internodes: Average length slightly more than one inch, which is about the length of an average Rotundifolia internode.

Leafbuds: Small, dark reddish-brown in color; mature buds more or less conical in shape; immature buds flattened like those of Rotundifolia.

Shoots: Leaves not expanding as rapidly as in Herbemont, but not as slowly as in Rotundifolia.

Color of Foliage: Almost green but slightly tinged with brown. This is especially noticeable on the tendrils, petioles and leaf veins and in the seedling stage on the hypocotyl and on the lower surfaces of the

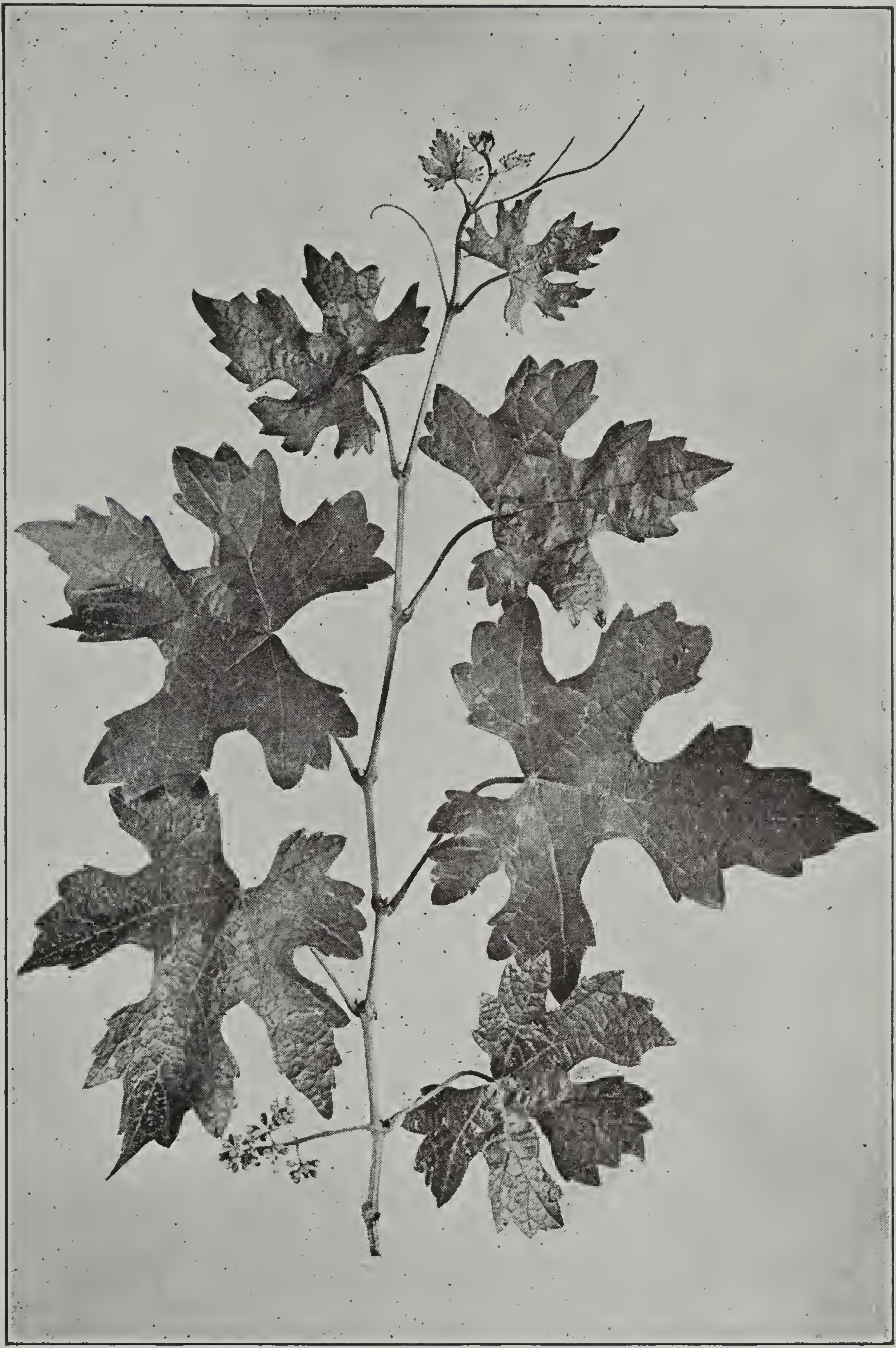

Fig. 11. A leafy shoot with a flower-cluster from the 1914 staminate Bourquiniana-Rotundifolia hybrid (Herbemont × Light Male No. 2). This is the oldest known Bourquiniana-Rotundifolia hybrid vine. Notice the five-lobed leaves which are characteristic of these hybrids; the short, simple and bifid tendrils, the small flower-cluster (similar to an average staminate Rotundifolia flower-cluster) and the slender and nearly straight cane. Reduced.

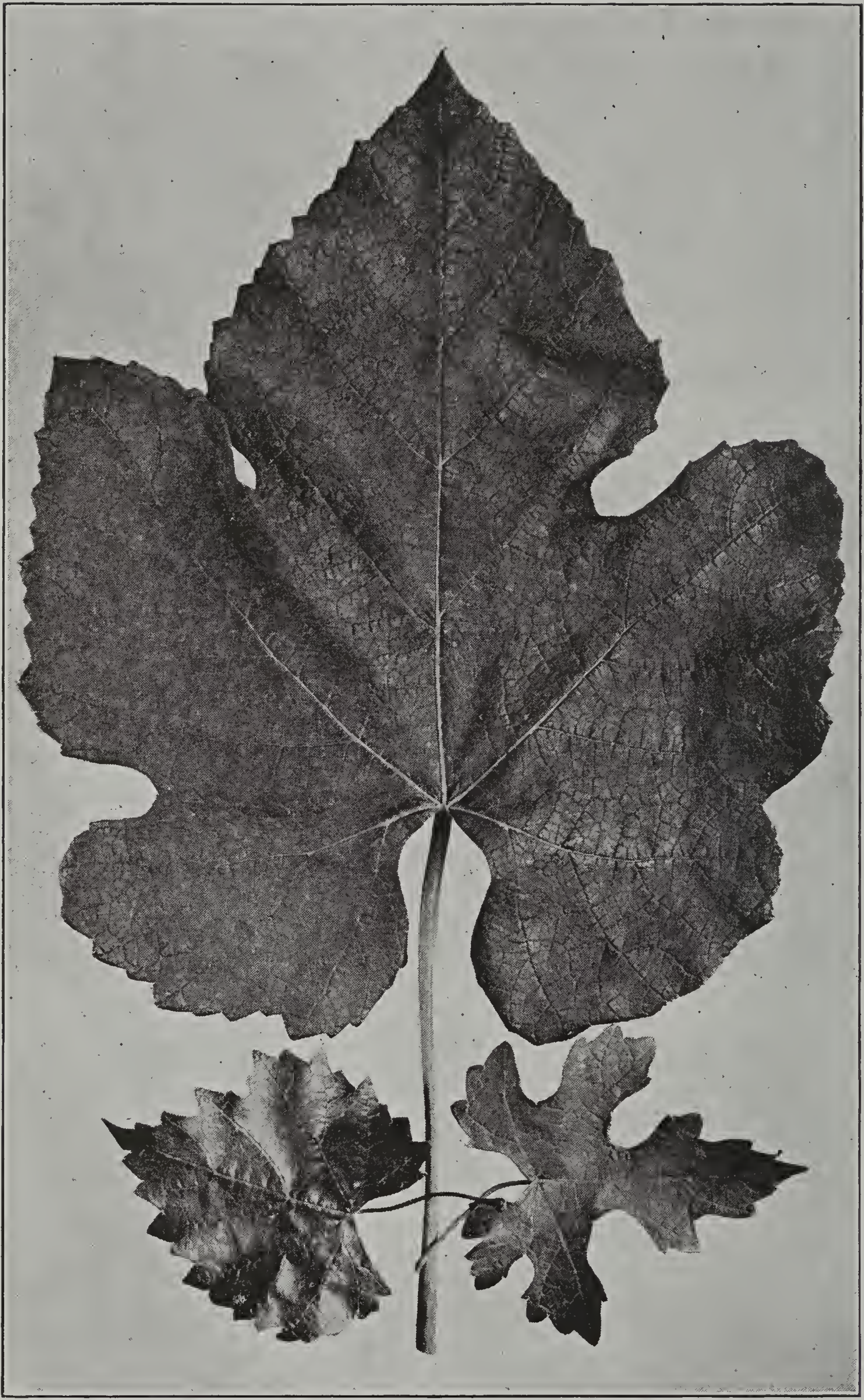

Fig. 12. Three typical leaves representing both parent and hybrid. Upper leaf is from Herbemont (female parent), lower left is from Rotundifolia (male parent), lower right is from the hybrid. The size of the leaf on this hybrid is determined by the Rotundifolia parent but the lobing is much more pronounced than in the mature stage of the Herbemont parent, however, the juvenile leaves of Herbemont are deeply lobed. Reduced.

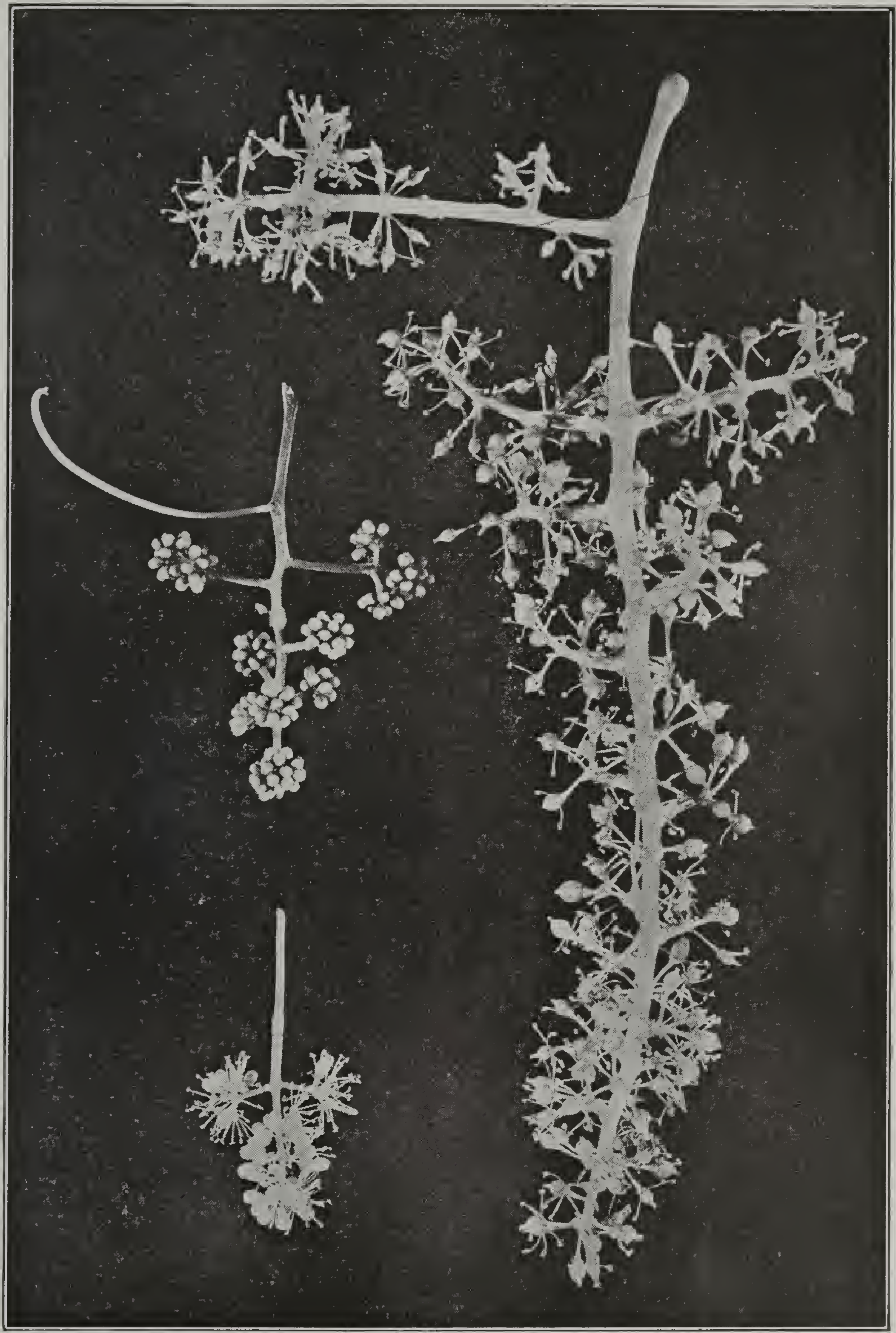

Fig. 13. A flower-cluster of each of the parents and of the hybrid vine. The large flower-cluster at the right is from Herbemont (perfect hermaphroditic but used as the female parent), upper cluster at the left is from a staminate Rotundifolia vine (male parent), lower cluster is from the 1913 hybrid vine (staminate). The size of the flower-cluster on this hybrid is determined by the Rotundifolia species. Reduced.

cotyledons. The nodes in young wood also carry marks of brown coloration. The young unfolding leaves are usually covered with a yellowish, occasionally slightly pink, pubescence.

Leaves: Small, the same size as that of Rotundifolia, deeply lobed into five, occasionally seven parts; teeth large, more like Rotundifolia than Herbemont; leaves silky hairy above and thinly pubescent beneath, especially along the petioles and veins; blades thin, glossy green and smooth above, paler beneath; stipules larger than in Rotundifolia.

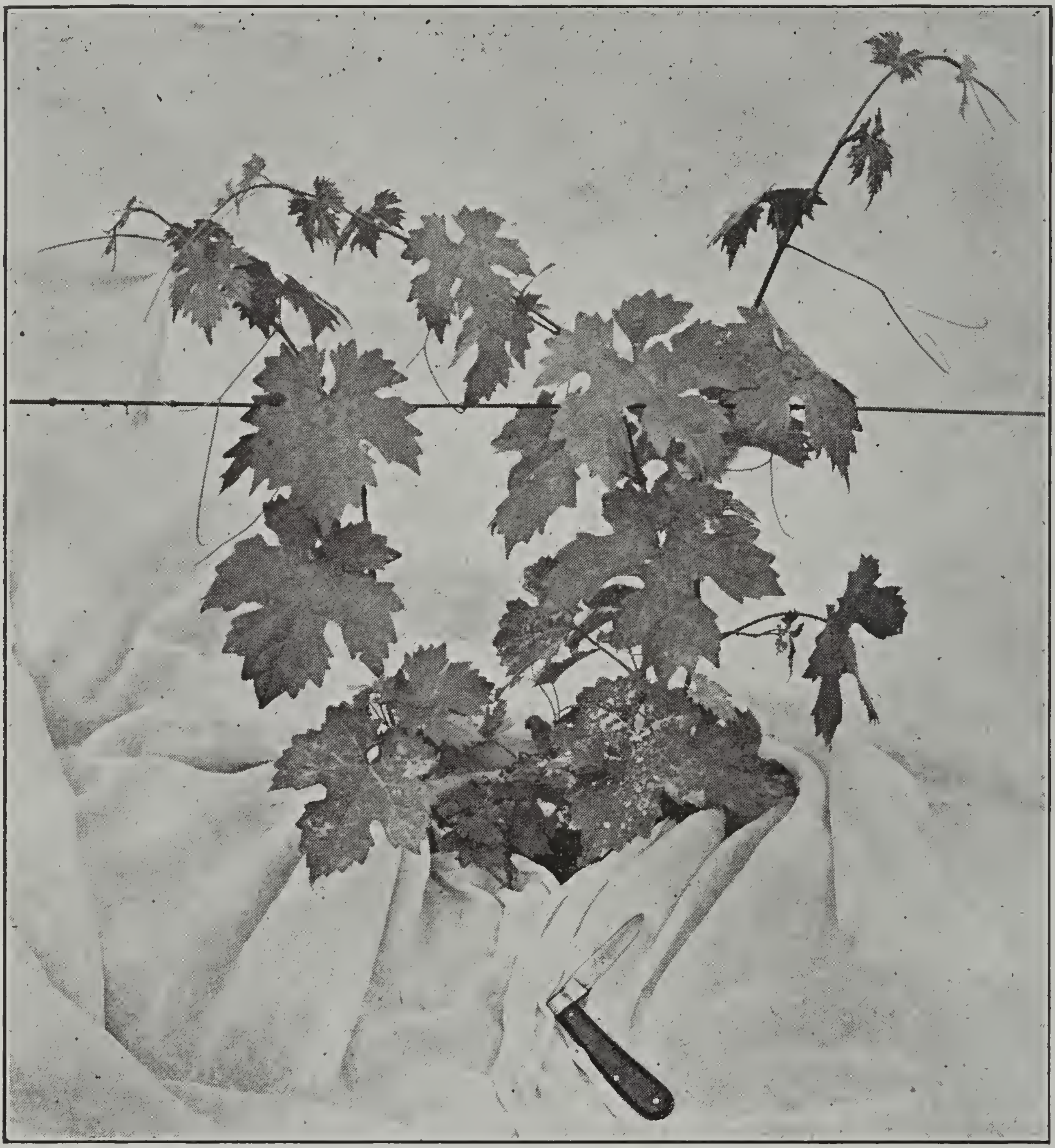

Fig. 14. A Vinifera-Rotundifolia hybrid vine (Malaga Seedling No. 1 × G-52) showing the character of growth, shoots, leaves, tendrils, etc. of this vine. Not all hybrids of this lot resemble the vinifera parent as closely as does this vine. Reduced.

Flower-clusters: Very small, similar to those on small clustered staminate Rotundifolia vines; peduncle and rachis not stout.

Flowers: Staminate on this vine; stamens upright; pistils almost entirely suppressed.

Blooming period: Late, practically simultaneous with that of Rotundifolia.

Fertility of the flowers: Since the vine is staminate, we can test only the pollen grains for fertility. A microscopic examination reveals a mixture of mostly shriveled and angular grains, but also some that are perfectly normal in shape. Pollination tests indicate that some of the pollen grains are fertile. The pollen is mostly sterile because of hybridization. As this vine happens to be staminate, no description of fruits and seeds is possible.

II-b. HERBEMONT × ROTUNDIFOLIA (G-52), 1916 and 1917.

The only difference between these vines and the preceding one lies in the following characters:

1. The color of the growing vines, leaves, tendrils, etc. is a deep reddish-brown.
2. The leaves are variably lobed.
3. These vines undoubtedly will bear two types of hermaphroditic flowers similar to those of the Winchell-Rotundifolia hybrid lots of 1916 because the male parent is one of the identical vines, G-52. The blooming period of these vines might be more intermediate between the two parent species.
4. The perfect and the imperfect hermaphroditic vines of these two lots will probably bear fruit which will be described in a later publication.
5. These vines will also be more or less sterile because of hybridization.

III-a. MALAGA SEEDLING NO. 1 × ROTUNDIFOLIA (G-52), 1916.

General character of plants: Vines are somewhat variable. Some are indicative of tall climbers while others seem to be more of the spreading type. While the lobing of the leaves is somewhat variable, nevertheless, all possess typical hybrid characters. Not much branched.

Vigor of vine: Variable from very strong to very weak.

Canes: Long, slender, fairly strong, somewhat zigzag, few in number, strongly angled on tip of cane, more or less rounded on the base, compressed, obscurely striated.

Wood: Softer than Rotundifolia but harder than Vinifera; new wood greenish in color.

Pith: Green, filled with starch during the dormant season, mostly continuous, occasionally interrupted by a diaphragm. In comparison with the diameter of the cane, the pith column is larger than in Rotundifolia and smaller than in Vinifera. At the nodes the pith cells are more compressed than elsewhere.

Bark: Nearly smooth, light grayish-brown in color; lenticels few, submerged, inconspicuous. Bark inclined to be shreddy even on young canes, no bloom.

Nodes: Enlarged on bud side.

Internodes: Somewhat longer than in Rotundifolia and much shorter than in Vinifera.

Leafbuds: Small to medium, conic on old wood and flattened on the younger wood, light brown in color.

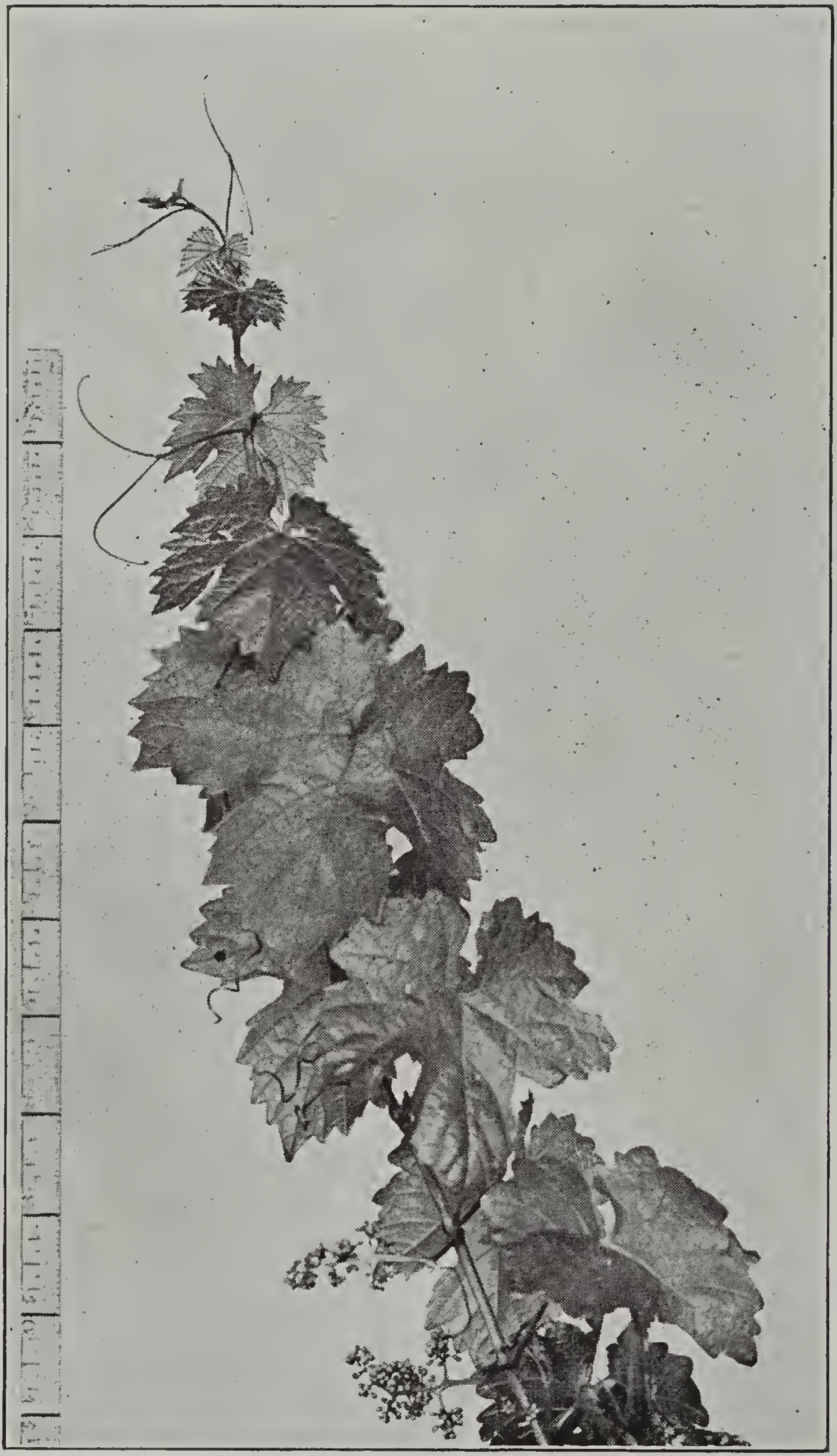

Fig. 15. A leafy shoot with flower-clusters from a Vinifera-Rotundifolia hybrid vine, showing the comparative sizes of the flower-cluster, leaf and tendril; also a different type of lobing of the leaves from that of Fig. 14. Reduced.

Shoots: Rapidly elongating, inclining toward nakedness.

Color of foliage: Light brown to reddish-brown. This is especially noticeable on the rapidly elongating canes and on the tendrils. The

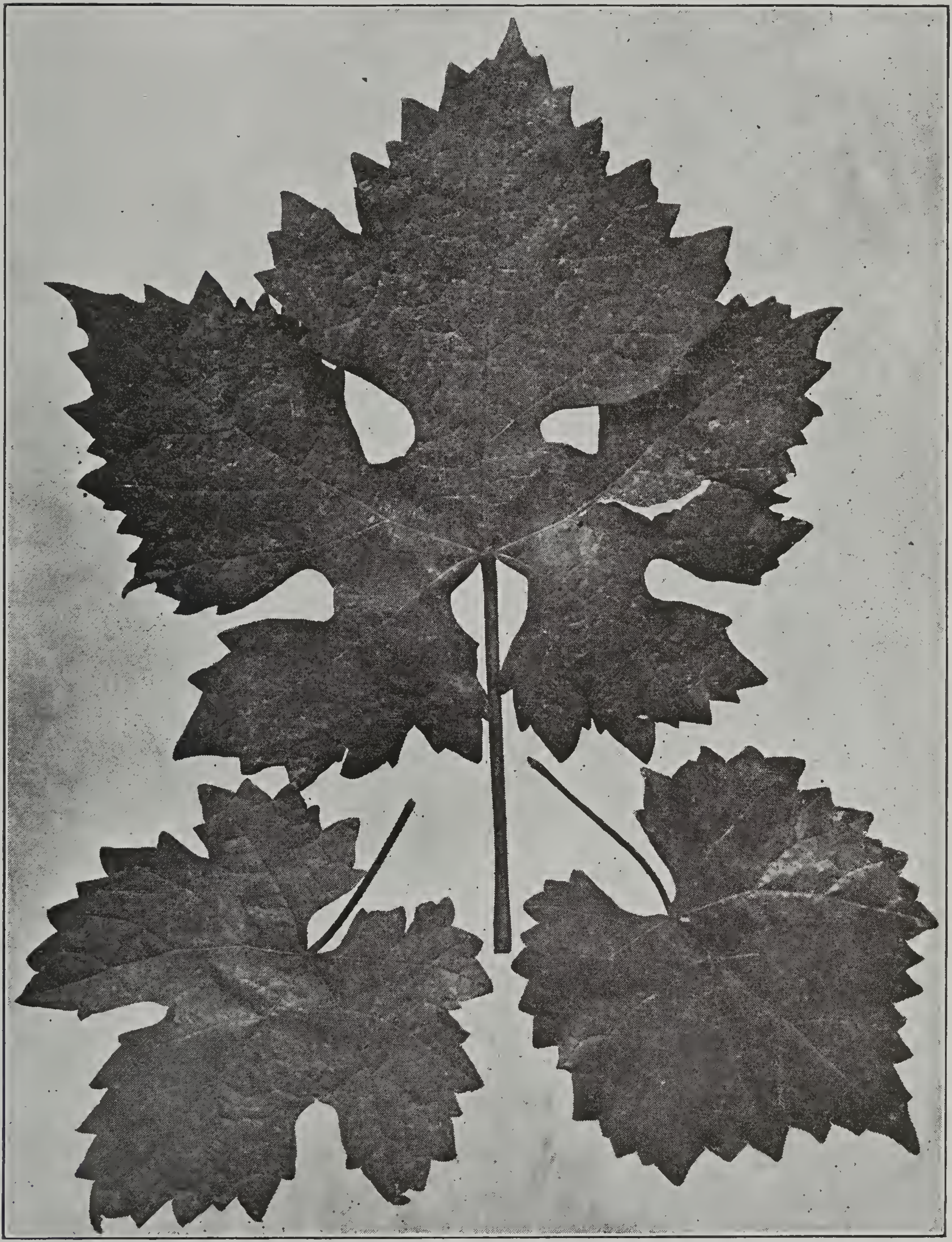

Fig. 16. Leaves from V. vinifera, V. rotundifolia, and from a hybrid vine. Upper leaf is from Malaga Seedling No. 1 (female parent), lower right-hand leaf is from a Rotundifolia vine (male parent), lower left-hand leaf is from a hybrid vine. The size of the hybrid leaf is largely determined by the Rotundifolia species while the character and the lobing seem to be more or less intermediate between that of both the parents. Reduced.

nodes usually are darker than the internodes. Expanding leafbuds and young leaves are covered with a slight whitish or pinkish pubescence.

Leaves: Small to medium in size, averaging pretty closely with Rotundifolia, sometimes a trifle larger, three to five-lobed on some vines and almost entire on others; upper surface of leaves smooth and dull green, silky when young, soon becoming smooth; lower surface paler green and slightly villous epecially along the ribs and veins; teeth large; stipules larger than those of Rotundifolia.

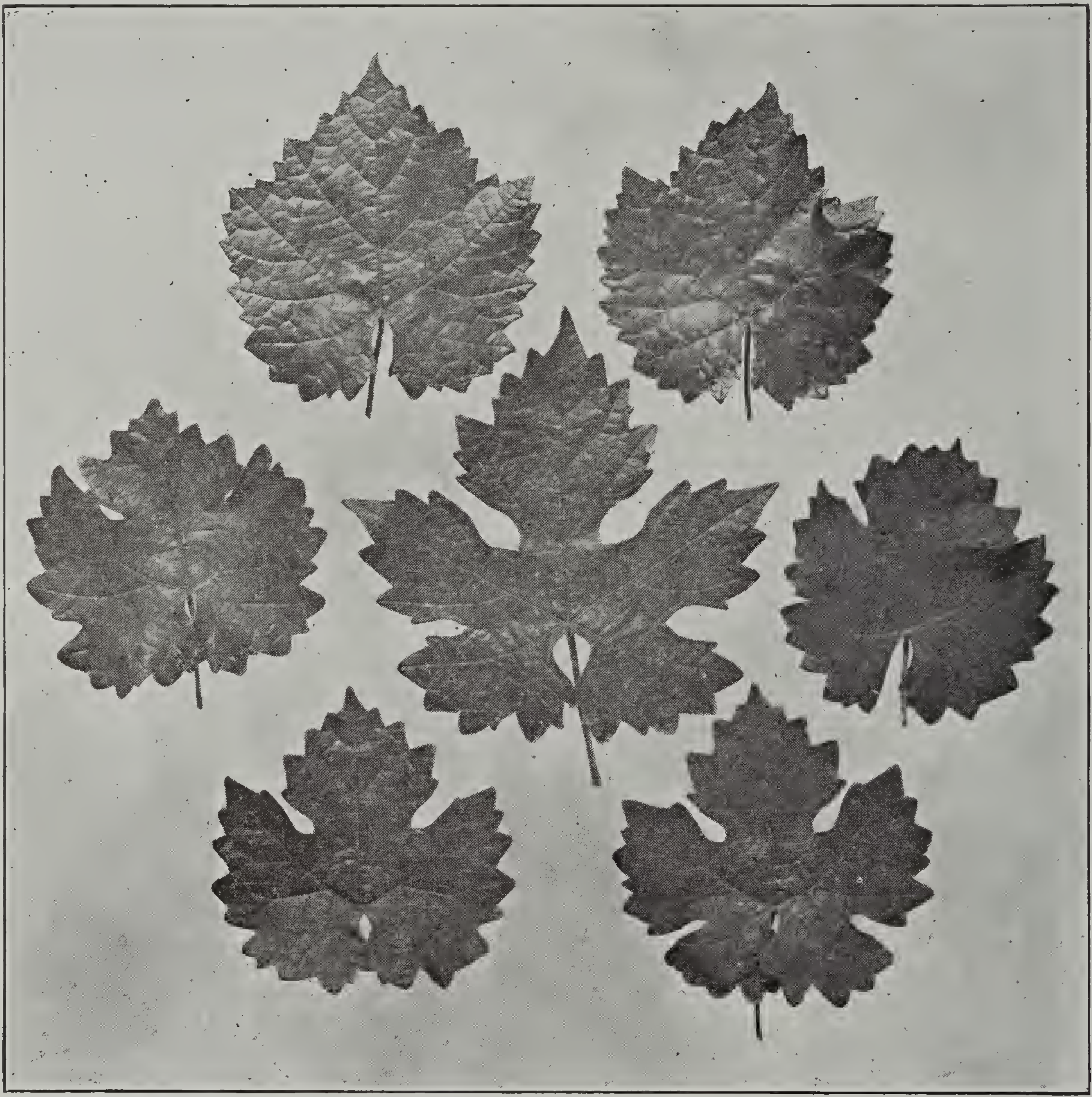

Fig. 17. A sample of leaves from the Vinifera-Rotundifolia hybrid vines, showing the differences that can be observed in the lobing of the leaves. Some are almost like Vinifera while others are almost like Rotundifolia leaves. Reduced.

Tendrils: Variable from simple to bifid, some are weak while others are strong, usually brown or reddish in color.

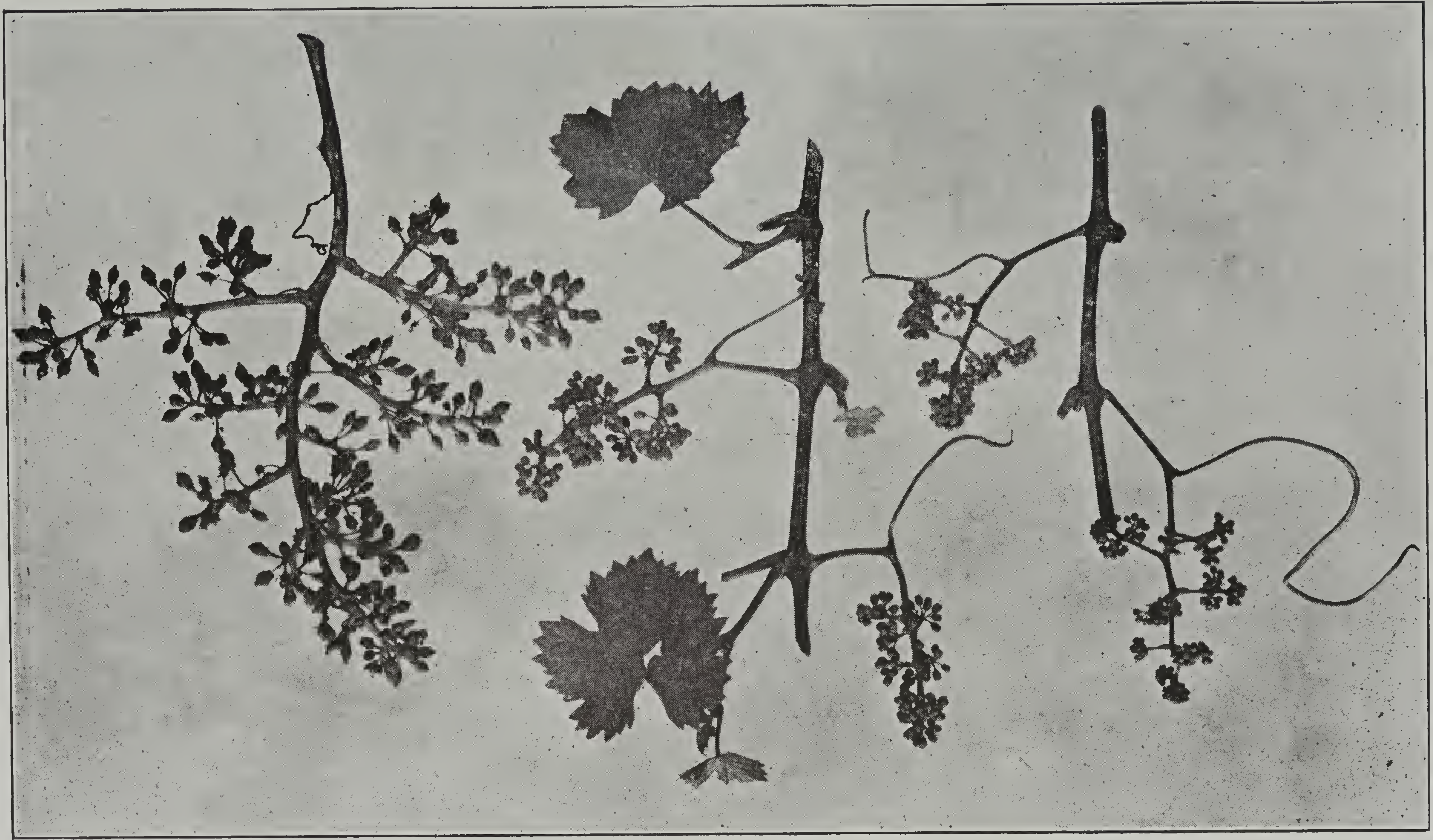

Fig. 18. Typical flower-clusters of V. vinifera, V. rotundifolia and of a hybrid vine. The cluster at the left is from Malaga Seedling No. 1, imperfect hermaphroditic (female parent) ; the one at the right is from G-52, perfect hermaphroditic (male parent) ; the one in the center is from a hybrid vine, perfect hermaphroditic. The small size of the flower-clusters of this and similar hybrid vines is determined largely by the Rotundifolia species. Reduced.

Flower-clusters: Two types as regards size; the clusters on the perfect hermaphroditic vines are small, practically the same size as those found on the Rotundifolia parent vine. The largest cluster produced about 300 flower buds. The clusters usually assume the characters of the Vinifera parent but the size and occasionally the branching of the Rotundifolia. The clusters on the imperfect hermaphroditic vines are much smaller and similar to those on the imperfect hermaphroditic Rotundifolia vines.

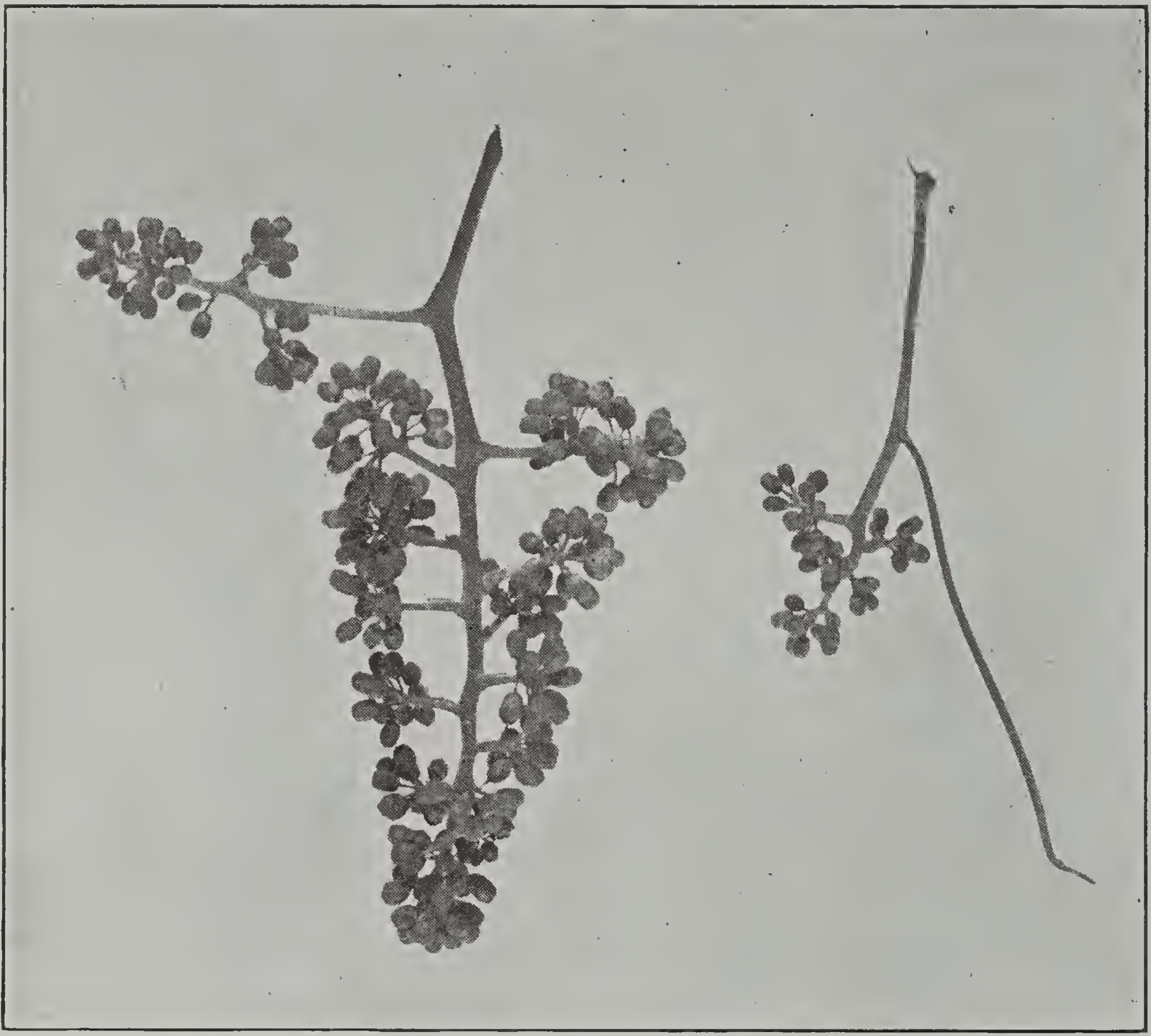

Fig. 19. Two types of flower-clusters found among the F_1 hybrid vines when Malaga Seedling No. 1 (imperfect hermaphroditic) is crossed with G-52 (perfect hermaphroditic). The cluster on the left (perfect hermaphroditic) and the cluster on the right (imperfect hermaphroditic) are typical in size of similar flower-clusters found among vines of the Rotundifolia species, otherwise the clusters are hybrid in character. Reduced.

Flowers: Perfect hermaphroditic and imperfect hermaphroditic; stamens upright and pistils medium large in the perfect hermaphroditic, stamens reflexed and pistils well developed in the imperfect hermaphroditic flowers.

Blooming period: Practically half way between that of the two parent vines.

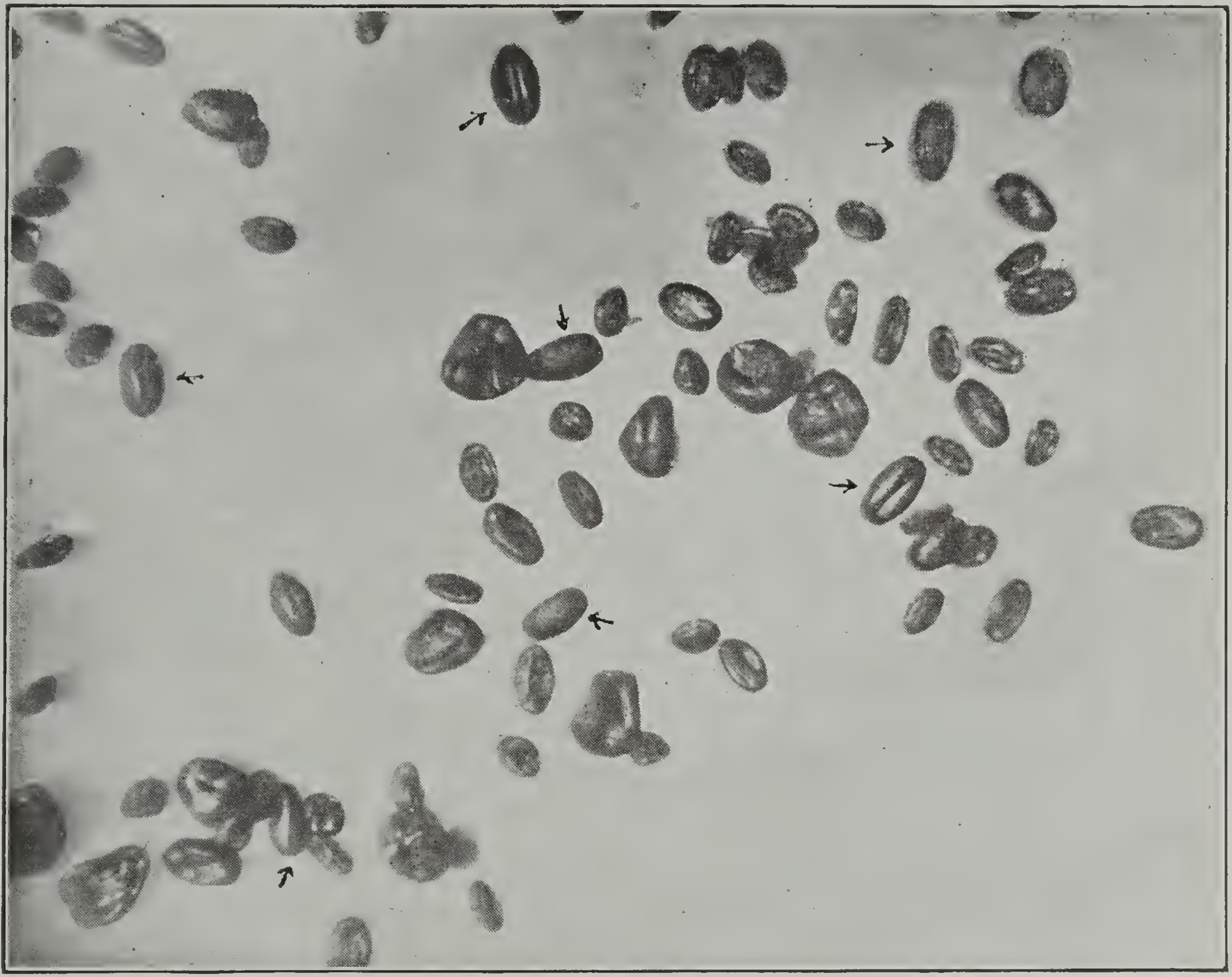

Fig. 20. A sample of the pollen that is produced by the perfect hermaphroditic hybrid vines of Vinifera-Rotundifolia parentage. Notice the comparatively few, about 14.4 per cent, normal pollen grains marked with an arrow. Magnified 230 diameters.

Fertility of flowers: The pollen in the perfect hermaphroditic flowers is a mixture of shriveled and plump, sterile and fertile grains. The fertility of these plump grains has been demonstrated in actual hand-made cross-pollinations, also by selfing some of the flowers. The pollen in the imperfect hermaphroditic flowers is all shriveled and impotent.

The pistils in both types of flowers are mostly sterile, only 2 from 17 perfect hermaphroditic flower-clusters having developed into berries in 1918. The perfect hermaphroditic flowers are sterile because of hybridization, while the imperfect hermaphroditic flowers are sterile due to the double phenomenon of hybridization and intersexualism with attendant impotence.

Fruit-clusters: Very small, only two berries have been grown to maturity and these were on one cluster. The results of 1918 indicate that the fruit-cluster will greatly resemble that of the Rotundifolia parent.

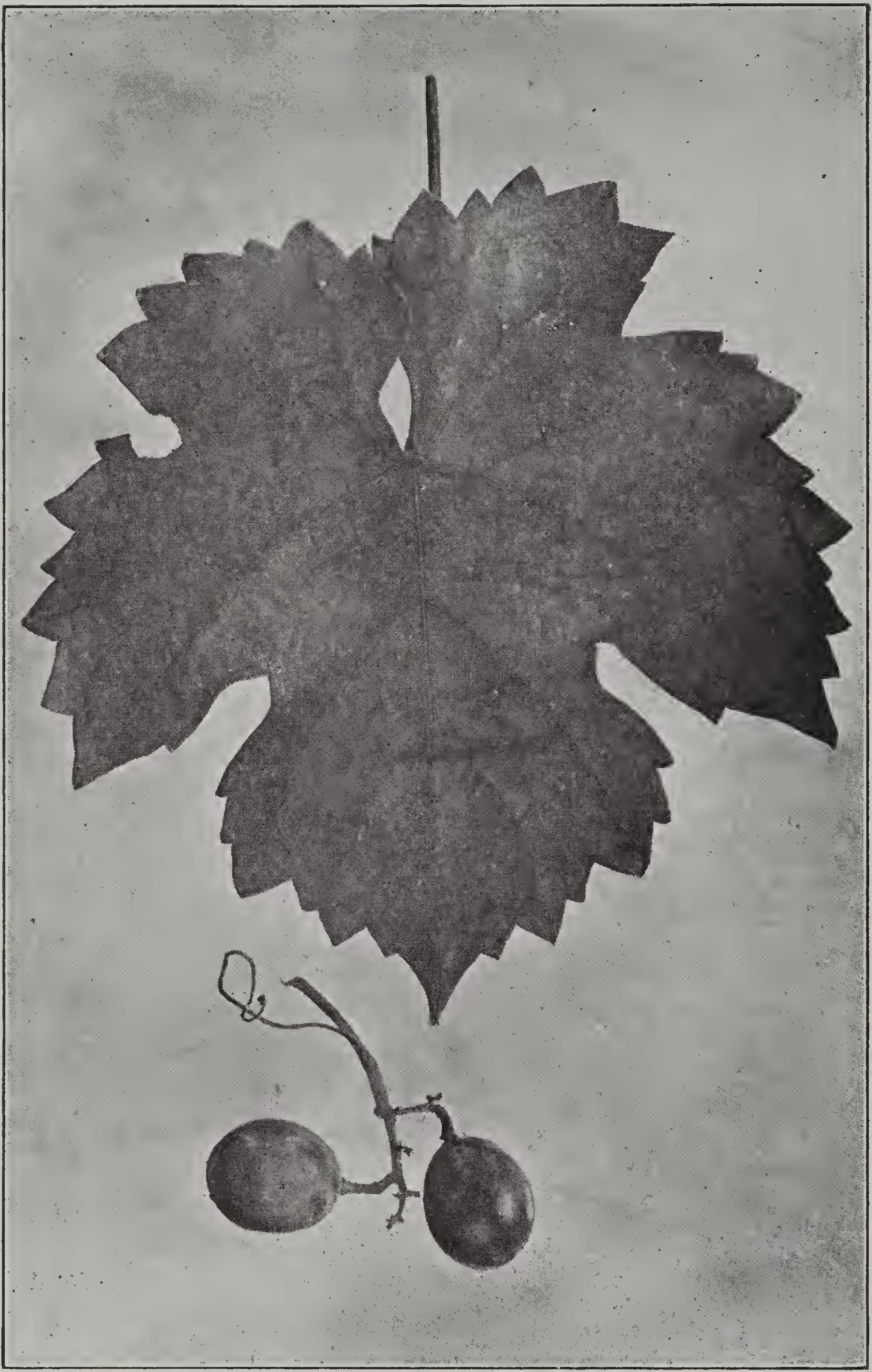

Fig. 21. A leaf and a fruit-cluster from a Vinifera-Rotundifolia hybrid vine (Malaga Seedling No. 1 × G-52). Notice the small fruit-cluster which is characteric of the Rotundifolia species. Natural size.

Berries: Medium in size, 15 mm. long and 13 mm. wide. These measurements have been taken of fruits from only one vine. Berries do not cling as firmly to the pedicles as do those of V. vinifera, but neither

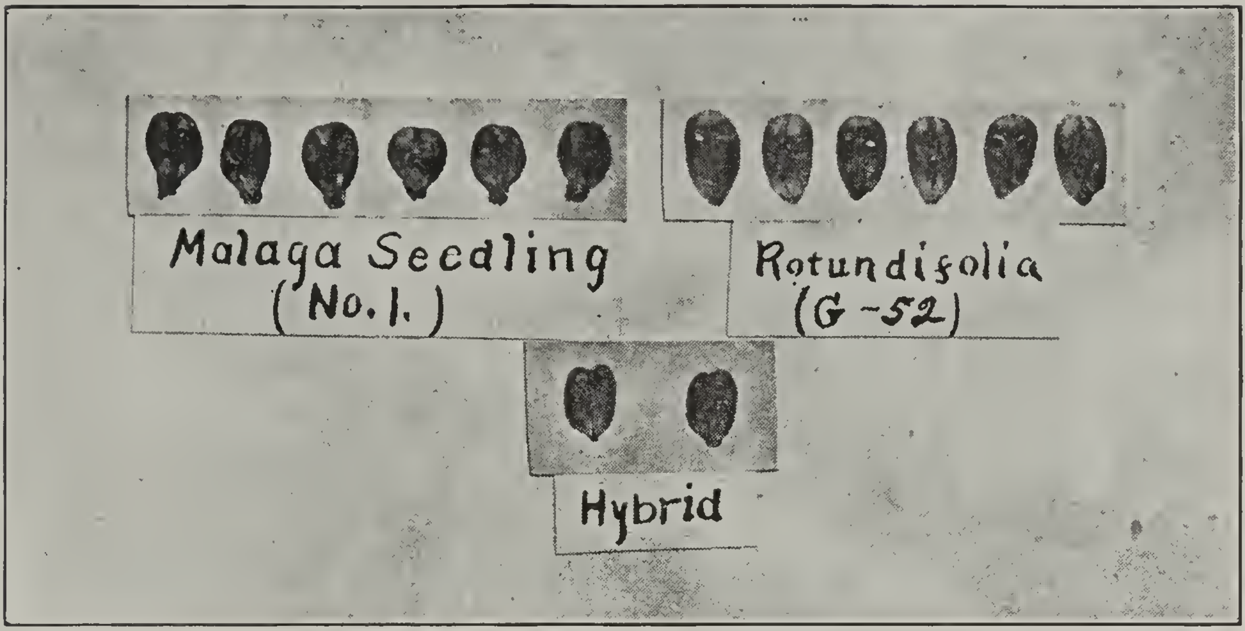
Malaga Seedling (No. 1.)
Rotundifolia (G-52)
Hybrid

Fig. 22. Seeds from the parent vines and from one of the hybrid vines. Malaga Seedling No. 1 (female), G-52 (male). The general outline and appearance of these hybrid seeds are largely determined by the Rotundifolia species. Natural size.

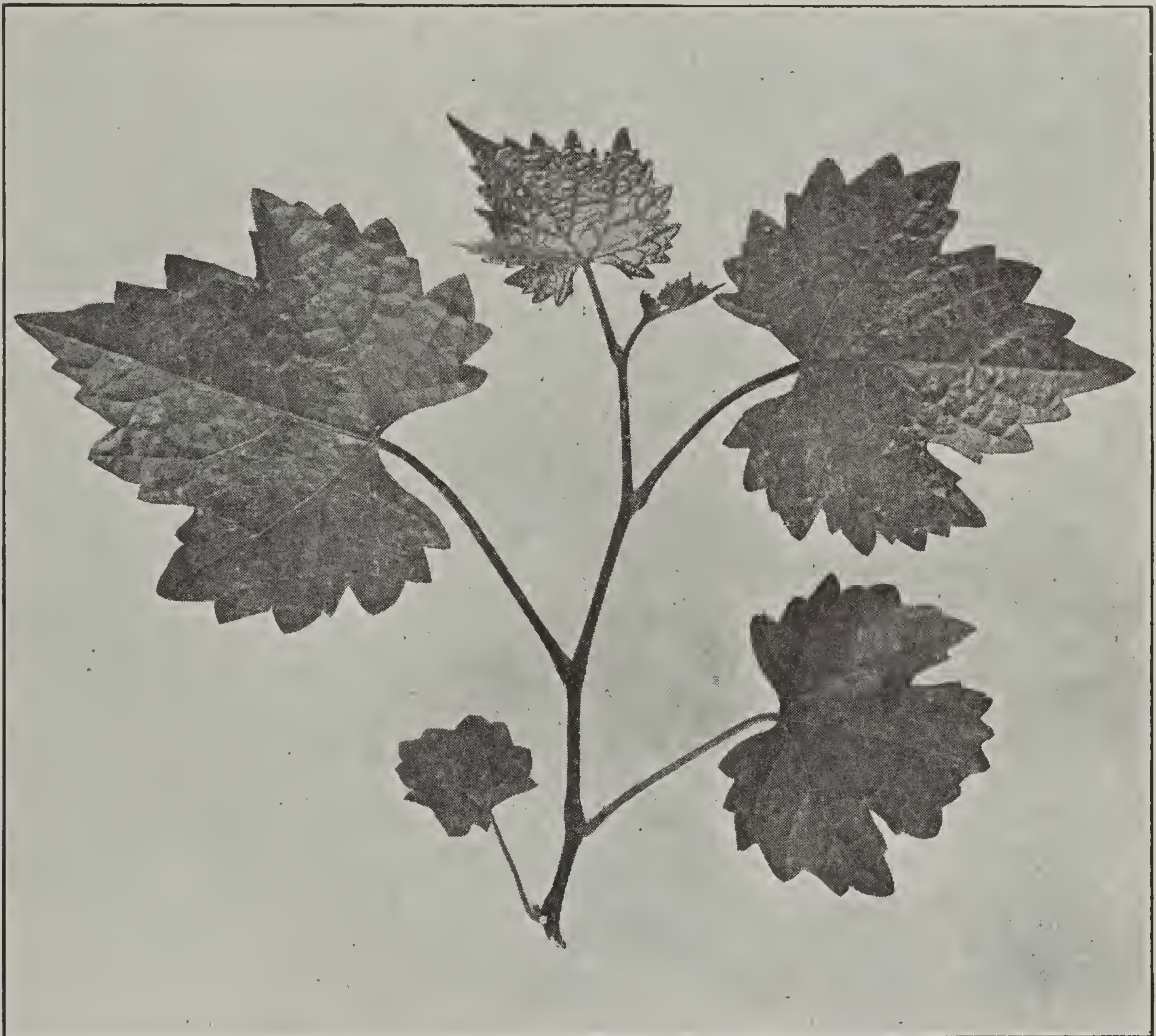

Fig. 23. A leafy shoot from a Rotundifolia-Vinifera hybrid vine (Oberlin vine as female and Malaga as male). Neither of the parent vines has a decidedly lobed leaf. Reduced.

do they shatter readily. The berries from this vine when fully ripe are black with a good coat of blue bloom, oblong in shape, skin thin but tough; flesh firm like in Vinifera; flavor sweet and vinous.

Seeds: One to each berry. 6.3 mm. long, 4.1 mm. wide, 2.9 mm. deep; beak obtuse. The color of the seed is dark chestnut brown with a light red beak and ventral depressions, glossy surface with minute, raised, light-colored specks; chalaza rather above the middle, sunken in both of the seeds, ovate to oblong in shape; suture deep and broad from chalaza over the top of the seed, not so deep and not so broad from the beak to the chalaza, slightly wrinkled around the chalaza; raphe very fine and hair-like, inconspicuous, extending from beak to the top of the seed and then lost in the suture; ventral depression shallow, broad, wrinkled on outer margins like Rotundifolia and lighter in color than the body of the seed; general outline of seed is that of Rotundifolia.

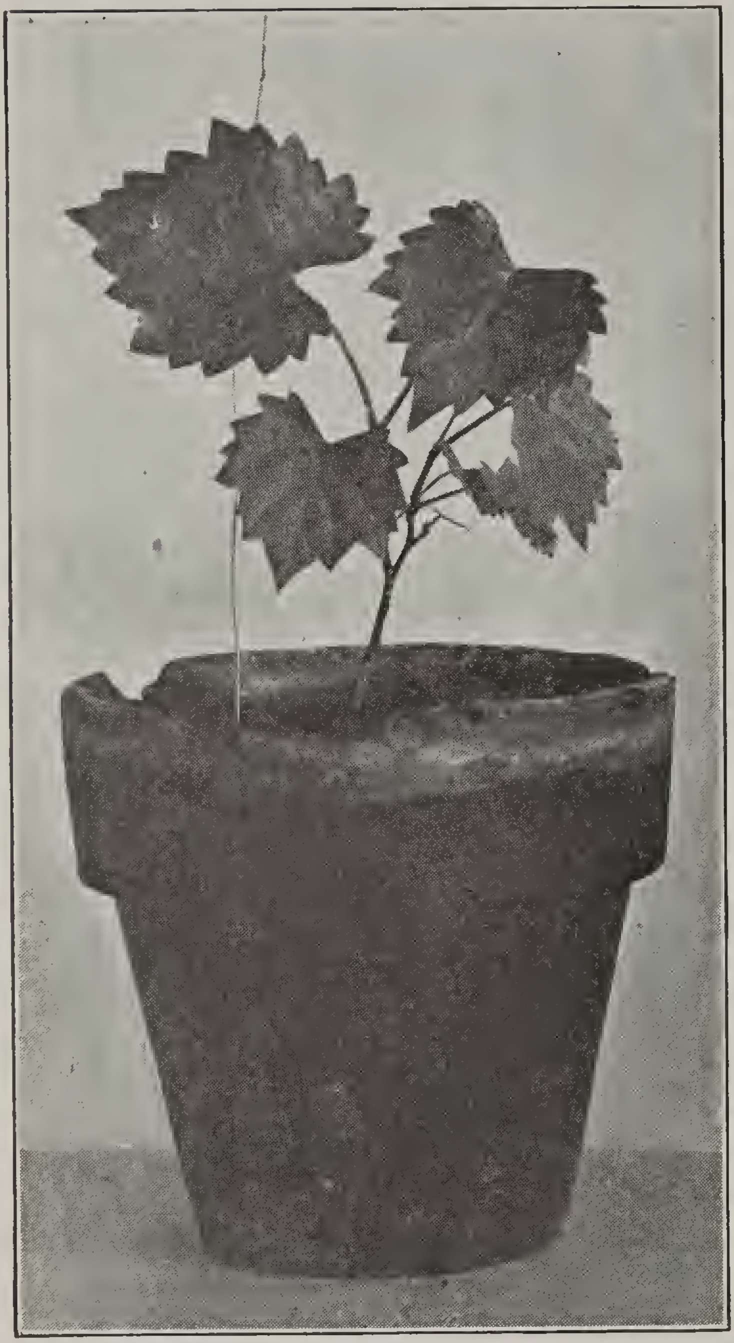

Fig. 24. A hybrid seedling (in a three-inch flower pot) of Oberlin vine when crossed with pollen from Malaga. The leaves of this vine are more like Rotundifolia than are those of the preceding. This vine is beginning the second year of its existence. Reduced.

III-b. Malaga Seedling No. 1 × Rotundifolia (I-1), 1916.

These hybrid seedling vines are very similar to those of the preceding. The only difference lies in the vigor of the vines, these being very weak.

IV. Malaga Seedling No. 3 × Rotundifolia (I-1), 1917 and
V. Malaga × Rotundifolia (G-52), 1917.

These hybrid seedling vines are only one year old and in all of their visible characters they are similar to the preceding.

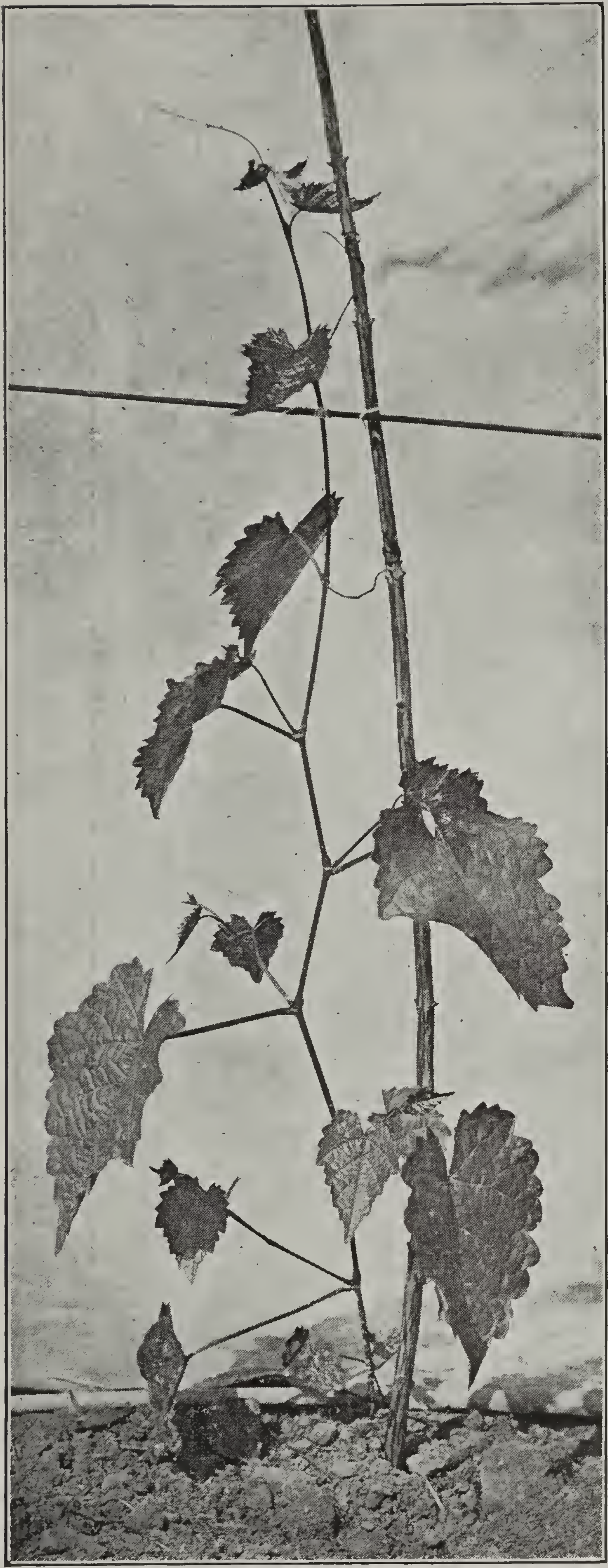

FIG. 25. An F_1 hybrid between V. cordifolia (female) and V. rotundifolia (male), 1918. Two existing vines of this type are the first hybrids to be recorded between these two species of grapes. The character of the growth is very much that of the female parent species. Reduced.

VI. OBERLIN VINE × MALAGA, 1917.

Vines one year old from seed.

Stems and bark are hybrid in character. The leaves are thin, light green, smooth and glossy above, paler beneath and villous especially along the veins and petiole, variably lobed; teeth large.

This description of the Rotundifolia-Vinifera hybrids is not extensive but it is sufficient to point out some of the hybrid characters of the vines.

VII. VITIS CORDIFOLIA × VITIS ROTUNDIFOLIA (I-1), 1917.

This description is taken from one-year-old vines. It will be only general in character.

Stems (first year): Very slender, dark red in color; leaves small, dark green above, paler beneath with pubescence along the veins, slightly silky when young, acuminate; pith mostly continuous. The growing habit of these vines is similar to that of Cordifolia seedlings. The vines are too small for minute description at this time.

VIII. CONCORD × ROTUNDIFOLIA (G-52), 1917.

Stem: Distinctly hybrid in character; bark persistent; leaves dark green, slightly wrinkled above, paler and cobwebby beneath. The shape of the leaves is very much like that of Concord.

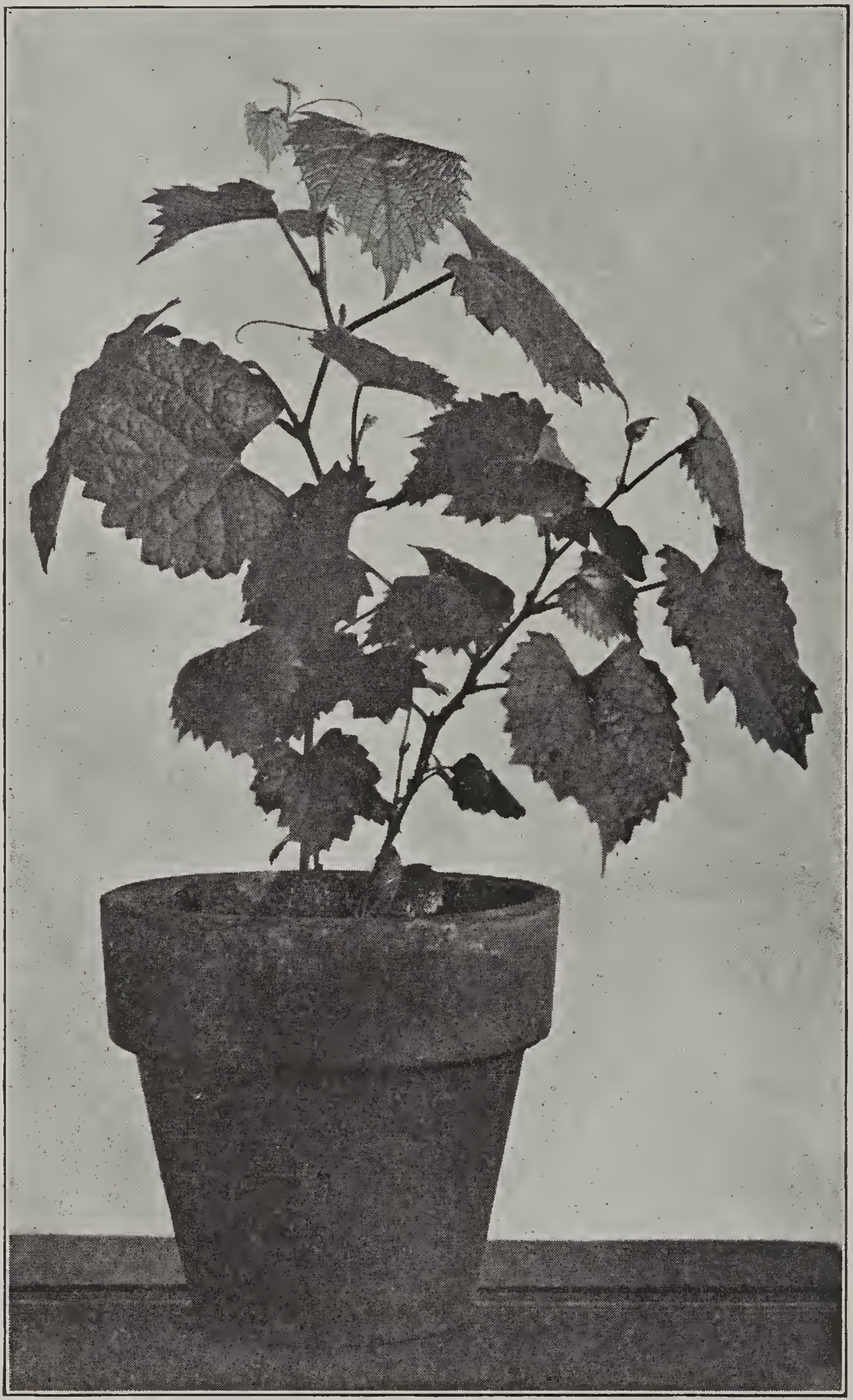

Fig. 26. An F_1 hybrid vine between V. labrusca, var. Concord (female) and V. rotundifolia, G-52 (male). The vine is in a three-inch flower pot and going on to its second year of growth. Notice the two simple tendrils, a bifid tendril is on the other side of the vine but not visible. Reduced.

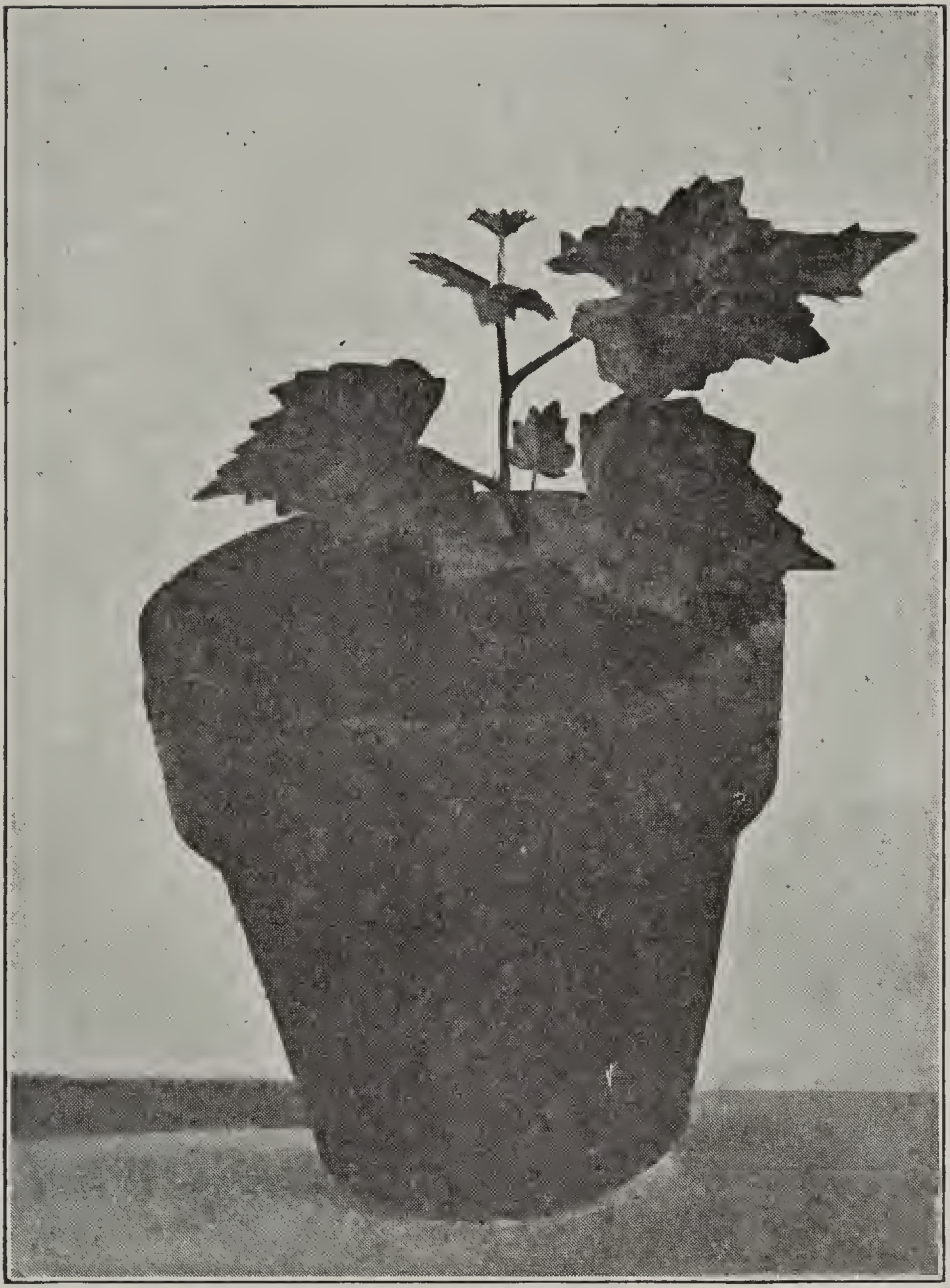

Fig. 27. An F_1 hybrid vine between V. aestivalis (female) and V. rotundifolia, G-52 (male). This vine has been grown from seed that germinated in March of 1919. The Rotundifolia leaf characters are plainly visible in this vine. Reduced.

IX. Winchell-Rotundifolia Hybrid × Rotundifolia, 1917.

The leaf and stem characters of this plant during the first season of its growth appear very much like those of the F_1 hybrid vine between Winchell and Rotundifolia.

C. A GENERAL SURVEY OF THE HYBRID CHARACTERS IN THE F_1 GENERATION

After having minutely described the characters of hybrid vines which are of Euvitis-Rotundifolia parentage, let us briefly compare these with similar characters that are to be found in the parental vines.

The canes may be said to be intermediate in character between the two types of parental vines in that they are nearly straight, somewhat angled like in Rotundifolia and more or less rounded, somewhat bent at the nodes and partially striated as in the Euvitis parents.

The wood is intermediate in color and hardness and bench-grafts grow readily.

The pith is more like that of Rotundifolia than that of Euvitis, but the occasional diaphragms and the indications of such structures at the nodes, also the occasional brown, dead pith cells without starch grains are characteristic of Euvitis.

The bark on both the young and on the old wood is of an intermediate character although the persistence of this bark to the wood is more of a Rotundifolia character. In some hybrids (Vinifera-Rotundifolia 1916) the bark is inclined to be somewhat shreddy but not entirely like that of Euvitis.

The nodes may be said to be intermediate in character.

The leafbuds are probably slightly larger than in Rotundifolia but their shape, especially at maturity, more nearly approaches that of the Euvitis bud.

The young growing shoots of the hybrid vines generally are soon clothed with foliage, but at times a shoot grows more rapidly and appears more or less naked.

The leaves are hybrid in character. The size of the leaf is practically that of Rotundifolia, while the lobing and general appearance and the size of the stipules when compared with the sizes of the leaves are characteristic of that of Euvitis.

The tendrils are variable in size and forking. Some are small and weak while others are found to be large and strong. Some are simple, some bifid and all gradations between these two extremes, but none of the trifid type have been found.

The flower-clusters are typically Rotundifolia in size but more like Euvitis in character. The type of flower in the F_1 hybrid seems to be governed largely by the Rotundifolia species when it is used as the male parent, for example:

Herbemont (perfect hermaphrodite) × Rotundifolia (staminate) produced 1 hybrid vine (staminate).

Winchell (perfect hermaphrodite) × Rotundifolia (staminate) produced 1 hybrid vine (imperfect hermaphrodite).

Winchell (perfect hermaphrodite) × Rotundifolia (perfect hermaphrodite) produced hybrid vines 4 of which are perfect hermaphroditic and 1 imperfect hermaphroditic.

Malaga Seedling No. 1 (imperfect hermaphrodite) × Rotundifolia (perfect hermaphrodite) produced hybrid vines 4 of which are perfect hermaphroditic and 2 imperfect hermaphroditic.

The blooming period of the staminate and the imperfect hermaphroditic hybrid vines coincides more nearly with that of Rotundifolia than that of the Euvitis species, while that of the perfect hermaphroditic vines seems to be more intermediate, however, some overlapping occurs.

The average fruit-cluster, judging from the few that were produced, is smaller than that of either parent but in character more nearly approaching that of Rotundifolia.

The berries are hybrid in character, but the character of the pulp and the sweetness seems to tend toward the Euvitis parent.

The seeds from general appearances are typically Rotundifolia although the colors of the beak, sutures and body of the seed as well as the character of the sutures disclose the true hybrid nature of the seed.

After having made these comparisons, we learn that in the F_1 generation Vitis rotundifolia is not as dominant in character as it is generally supposed to be; that the hybrid vines are not characteristically Rotundifolia in type but more or less intermediate.

Munson (2), speaking about grapes in general, says, "In some combinations, all the characters of one parent are dominant and, of course, all others recessive. This is true of Vitis rotundifolia (as female) when united with varieties of true bunch grapes, the Rotundifolia character being dominant." In the same article he endeavors to establish a law governing the characters in the F_1 generation when a species of Vitis with variable characters is mated with another species the characters of which are very uniform.

The law laid down by Munson reads thus, "Species of grapes very uniform in character when hybridized with species of very variable character give progeny with the characteristics of the uniform species dominant." These conclusions drawn by Munson and based on his so-called "false hybrids" when compared with the results of similar work done at the North Carolina Experiment Station must be considered as incorrect.

D. SO-CALLED "FALSE HYBRIDS"

"False hybrids" defined: "False hybrid" is a term that has been repeatedly used by some early Rotundifolia grape breeders and may be defined thus: a hybrid whose chief visible characteristics have been imparted to it by one of the parents only and all or practically all of whose recessive characteristics have been imparted to it by the other parent.

Theoretically such a hybrid may be possible, several have been reported, but on critical examination these usually turn out to be nothing more than straight seedlings from one of the parent species.

"False hybrids" have been reported by Professor A. Millardet of France (1), by T. V. Munson of Denison, Texas (1) and Dearing (3) reports "One supposed hybrid was secured, a cross of Eden (female) and Flame Tokay (male)." This supposed hybrid from its description, meager as it is, can very easily be placed alongside the "false hybrids" of Millardet and Munson.

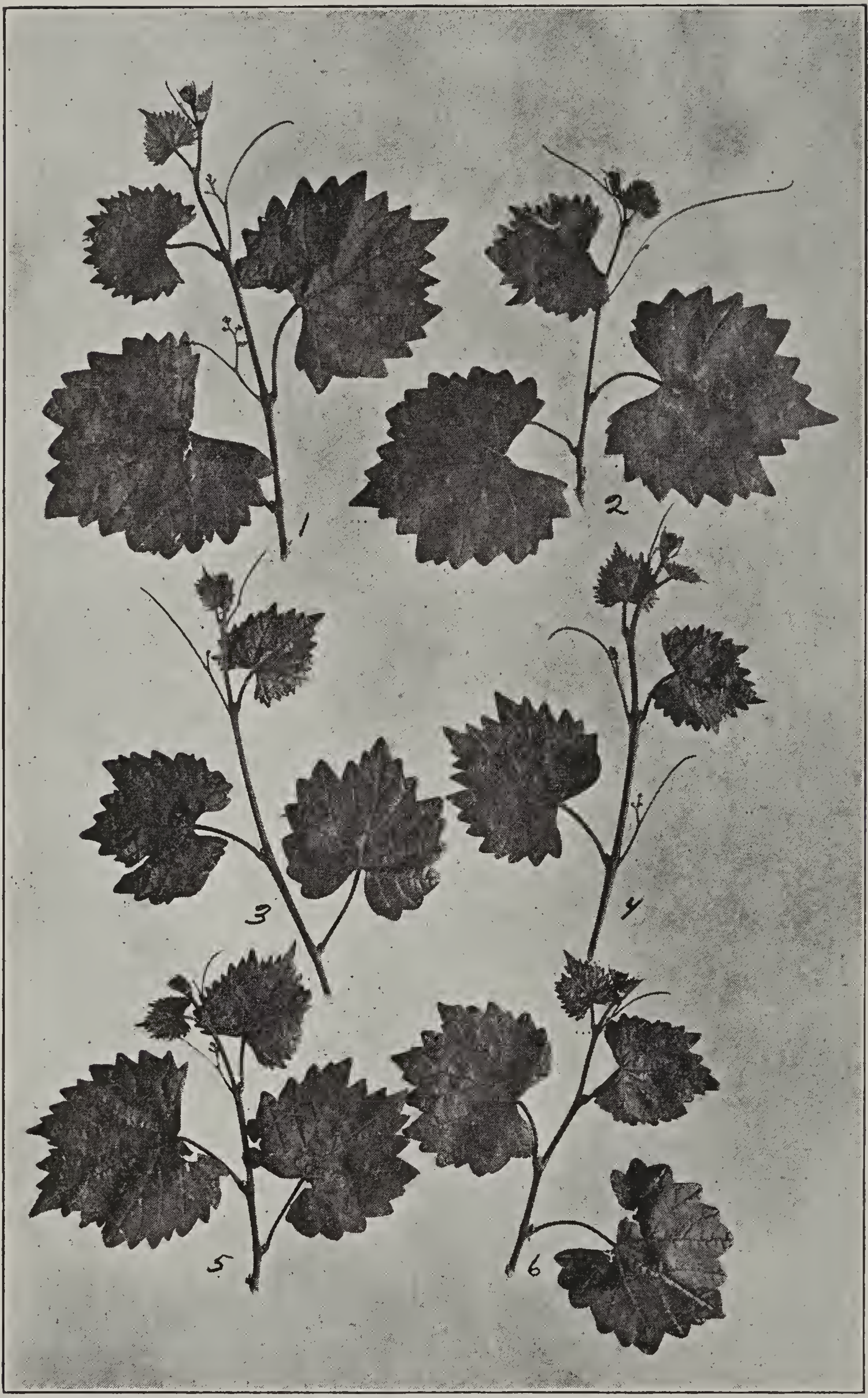

Fig. 28. Leafy shoots from six of Munson's so-called "false hybrids." Reading from left to right they are: (1) Sanalba, (2) Labama, (3) LaSalle, (4) San Jacinto, (5) San Melaska, (6) Sanmonta. Notice that all the characters of the tendrils, shoots and leaves are those of true Rotundifolia vines. Reduced.

"False Hybrids" and "True Hybrids" of Vitis Rotundifolia Compared

If the Rotundifolia-Euvitis hybrids were "false hybrids" then we should not be able to distinguish them from vines of the Rotundifolia species, they being identical in appearance; if, however, such hybrids be not "false hybrids" then we should readily perceive their intermediate characters. From the descriptions of the hybrid vines that were produced and grown by this station, we note that these differ from the vines of the Rotundifolia species in the following:

Wood: The wood of Rotundifolia vines is greenish in color, hard and difficult to graft while that of the hybrids is less green, less hard and less difficult to graft.

Pith: The pith of Rotundifolia vines is green, continuous and packed full of starch in the dormant stage while that of the hybrids is mostly green, sometimes sappy brown, mostly continuous but indications of diaphragms are seen at almost every node; starch is packed in the green and greenish-brown pith cells but is lacking, except for a few stray grains, in the brown pith cells. (See Figs. 3 and 4.)

Bark: The bark on Rotundifolia vines is of a light brown color, overlaid with a thin coat of gray and thickly dotted with gray surface lenticels. The bark is persistent on the young wood and on old wood it separates in broad plates or patches. In the hybrid the color may vary from light to a dark brown overlaid with a very thin coat of gray and the lenticels are mostly submerged and almost invisible. The persistence of the bark to the wood and the shedding of the bark in large plates or patches is in some cases practically that of the Rotundifolia parent, while in one case at least, viz.: Vinifera-Rotundifolia hybrid, these characters are practically intermediate.

Internodes and nodes: The internodes in Rotundifolia vines are short and the nodes are not enlarged, while in the hybrid vines the internodes are longer on the average and the nodes are somewhat enlarged on the bud side. (See Fig. 4.)

Leafbuds: The leafbuds on Rotundifolia vines are small, subconic and pubescent on top while on the hybrid canes they are more or less conical, almost smooth and much larger in size.

Shoots: The shoots of Rotundifolia vines are considered naked, while those of the hybrid when the other parent does not have similar shoots generally are clothed immediately with leaves. (See Figs. 5, 11, 14, 15, 23, 25, 26 and 29.)

Leaves: The leaves of Rotundifolia vines are small, generally not lobed but occasionally indications of lobing are noticed in the leaves of some vines, smooth and glossy above and below, large acute marginal teeth, pubescence only in the angles of veins on the lower side of the leaf. The leaves of the hybrid are small, very nearly like those of Rotundifolia, intermediate in lobing but sometimes lobed even more than those on the Euvitis parent, more or less intermediate in wrinkling of the surface and in gloss; marginal teeth are more intermediate in character and the pubescence is intermediate in amount and density. (See Figs. 6, 12, 16, 17, 23, 24, 25 and 26.)

Tendrils: Simple in Rotundifolia but occasionally bifid, varying in the hybrid from simple to strongly bifid, not generally simple. (See Figs. 5, 11, 14, 15, 25 and 26.)

Flower-clusters: Small in the staminate and perfect hermaphroditic vines and very small in the imperfect hermaphroditic vines of the Rotundifolia species; small in the staminate and perfect hermaphroditic vines and very small in the imperfect hermaphroditic vines of the F_1 hybrids. The Rotundifolia species in this case seems to practically dominate the size of the flower-clusters. Except for size, the flower-clusters seem more intermediate in character between the two parent species. (See Figs. 7, 9, 11, 13, 15, 18 and 19.)

Blooming period of vines: In the F_1 hybrids the blooming period seems to practically fall midway between that of the two parent species. The only exception to this we find in our 1912 Winchell-Rotundifolia hybrid and in our 1913 Herbemont-Rotundifolia hybrid whose blooming periods practically coincide with that of the Rotundifolia species.

Sterility of flowers: In the Rotundifolia species we find two types of flowers as regards sterility; the perfect hermaphroditic flowers may be said to be self-fertile, the staminate and imperfect hermaphroditic flowers are self-sterile, due to sex and intersexualism with attendant impotence. Among the flowers of the hybrid vines we find another type as regards sterility, namely, the one that is sterile because of hybridization. In the anthers of perfect hermaphroditic grape flowers we generally find normal pollen grains, but in the anthers of such flowers of the F_1 hybrids we find mostly irregular pollen grains; occasionally a normal grain is produced. (See Figs. 10 and 20.) Again the ovules in perfect hermaphroditic grape flowers generally may be said to be fertile, but in the F_1 hybrid the fertile ovule seems to be the exception, for out of all of the ovules from about twenty flower-clusters of this type of flower only two developed into fruit. The sterility of these ovules can easily be attributed to the hybrid origin of the vines. In the anthers of imperfect hermaphroditic grape flowers we generally find aborted pollen grains resulting from the phenomenon of intersexualism, but the ovules in such flowers are usually fertile. In the imperfect hermaphroditic flowers of the F_1 hybrids we find as usual aborted pollen grains in the anthers, but most of the ovules also are sterile: hence sterility in this case is due to the double phenomenon of intersexualism with attendant impotence, and hybridization.

In the anthers of our staminate hybrid vine, Bourquiniana × Rotundifolia, we again find mostly all aborted pollen grains, hence this vine also is practically sterile because of the double phenomenon of sex and hybridization. Sterility due to hybridization therefore seems characteristic of all Rotundifolia-Euvitis hybrids, and any vine that is claimed to be a hybrid but which is normally fruitful must in the light of our experiments be regarded as of nonhybrid origin.

Fruit-clusters: Small to very small in Rotundifolia and still smaller in the hybrid because of sterility due to hybridization. (See Fig. 21.)

Berries: Roughly speaking, the berries might be said to be of an intermediate character in the hybrid.

Seeds: The seeds of Rotundifolia are oval in general outline, the ventral sutures, extreme ends excepted, are generally parallel to each other. The seeds of the hybrid inherit the same general shape as is found in the Rotundifolia parent but the ventral sutures and some minor markings appear more like similar markings found in Euvitis. The size of the seed seems to be intermediate. (See Figs. 8 and 22.)

From the general comparisons drawn between our true F_1 hybrids and their supposed dominant parent species, Vitis rotundifolia, whose description should correspond with that of the so-called "false hybrids" we can safely conclude that the so-called "false hybrids" are no hybrids at all but simply straight seedlings of Vitis rotundifolia.

CONCLUSIONS

Turning now to the previously reported "false hybrids" we shall endeavor to show why these should not be considered as hybrids with Euvitis but as seedlings of the Muscadine group.

Professor Millardet reported all of his vines as "false hybrids." This information is gathered from a statement made by Dr. T. V. Munson, who had considerable correspondence with Professor Millardet. This statement taken from Munson's Foundation of American Grape Culture and found on page 208, reads as follows:

"The writer (Dr. Munson) had noted what Professor Millardet had written him about "false hybrids" including all his Scuppernong hybrids as such; that while they were actual hand-made hybrids, yet the Rotundifolia was so firmly fixed that its characteristics were the chief to show forth in the hybrid, those of the other parent being 'recessive' according to Mendel's characterization."

The vines of Professor Millardet were lost but this short, general but definite description of them seems, in the light of our investigations with hybrid material, sufficient to stamp them indelibly as being pure seedlings of the Muscadine group and not as hybrids, as their originator supposed them to be.

The varieties LaSalle and San Jacinto originated and disseminated by the late Dr. T. V. Munson at Denison, Texas, which are claimed to be hybrids between Scuppernong (female) and some Post-Oak × Herbemont hybrids (male); and his other similar varieties, Sanalba, Sanmelaska, Labama, Sanmonta and San Rubra, which are similarly claimed to be hybrids between the variety San Jacinto (female) and Brilliant (male), have been grown under constant observation on the college grounds for nine years and critically examined, and not one trace of hybridity between Vitis rotundifolia and Euvitis has ever been discovered. They are in all respects true Muscadine vines.

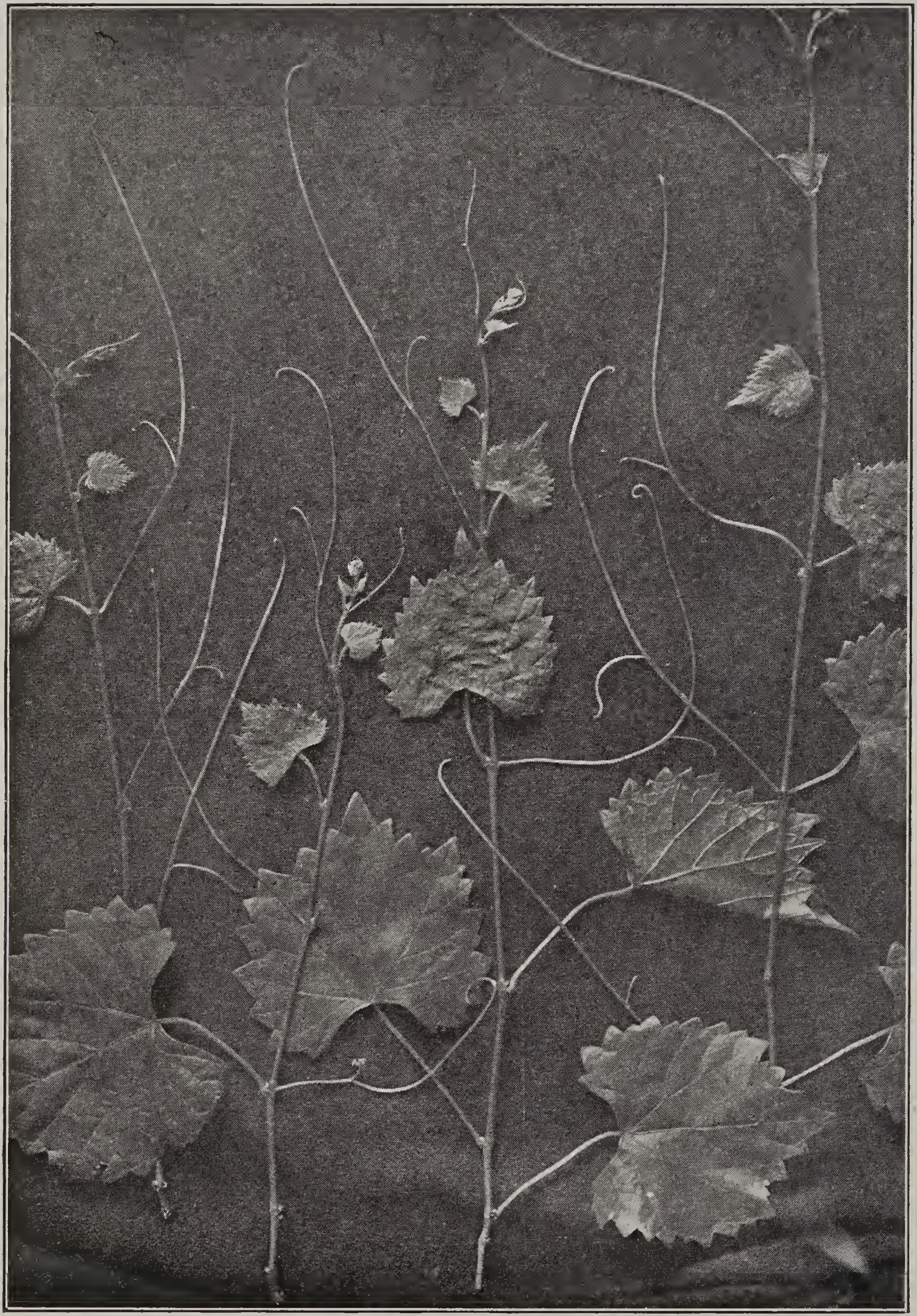

Fig. 29. Selected shoots from a Rotundifolia vine, variety Scuppernong. Notice that many of the tendrils are bifid. Reduced.

The characters on which Dr. Munson based the hybridity of these vines are such as may be found every once in a while among seedlings of pure Muscadines. The tendency toward lobing of the leaf has been repeatedly observed among seedlings of the variety Scuppernong, as well as other varieties. (See Fig. 30.) The occasional forking of the tendril has been noticed on two wild Rotundifolia vines as well as on numerous seedlings even more often than on any of his so-called hybrids. (See Figs. 29, 30 and 31.) The fruit-clusters on six of Munson's

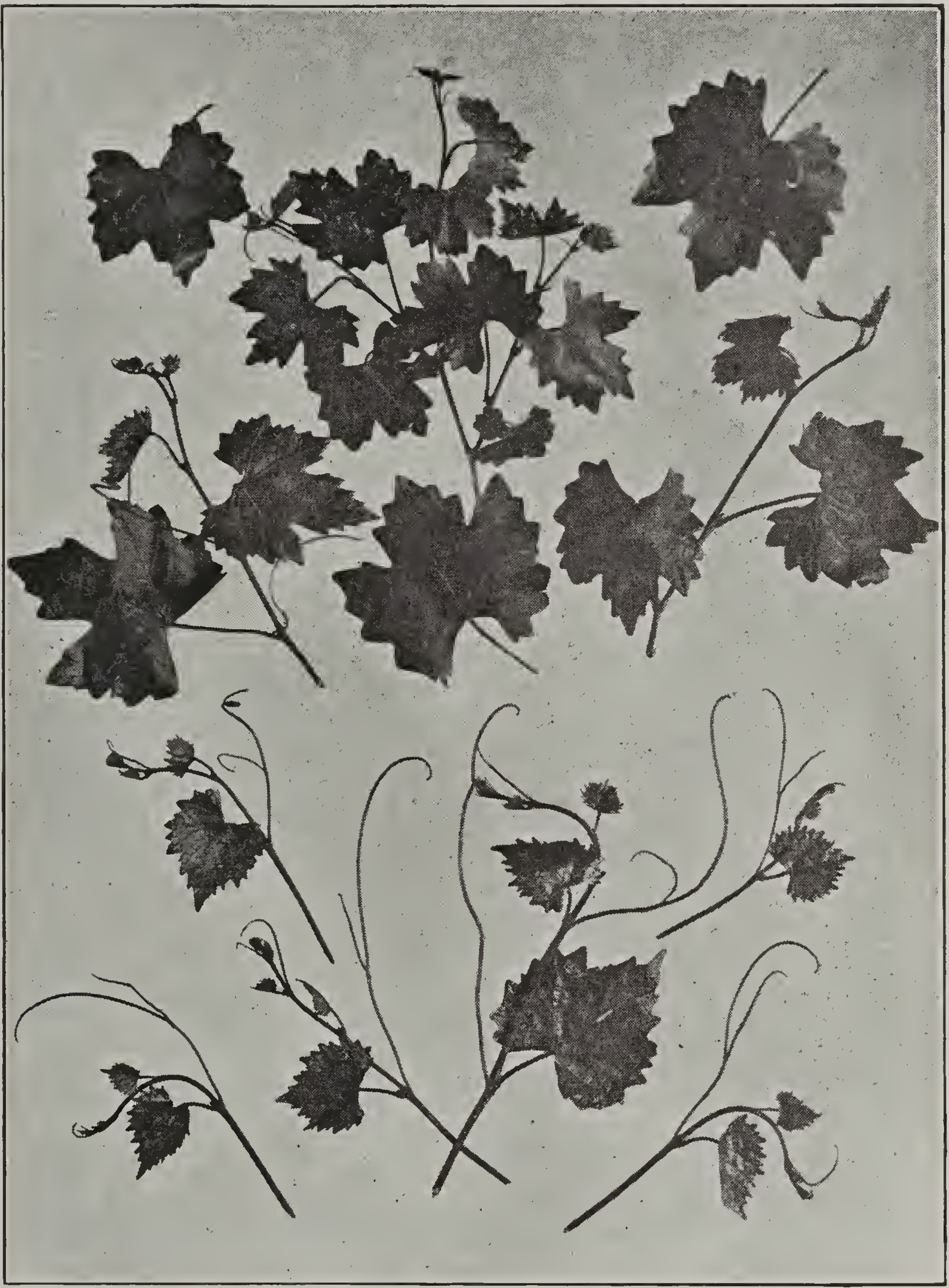

Fig. 30. Selected shoots from Rotundifolia seedling vines, showing variations. Leaves of upper shoots show a decided tendency toward lobing while the tendrils of the lower shoots show that all tendrils on Rotundifolia vines are not simple. Reduced.

hybrids are no larger than on many of our seedling vines of Scuppernong × Dark Male Vine No. 1 origin, while the fruit-cluster together with the size of the individual berries on his Sanmonta vine are only typical of those of Vitis munsoniana which species Dr. Munson had growing on his estate at that time. (See Figs. 32 and 33.) The thin skin, the character of the pulp, the seeds, the general quality of the fruit, the bark and other characters of Munson's vines are all to be found now and then in the Muscadine group. The sugar content of Sanalba has been far exceeded in our own Rotundifolia seedlings and the pink color of San Rubra has often appeared even more intense than this on light-colored seedling vines in our Muscadine nursery. Again Munson's vines are normally fruitful while from our work we learn that the F_1 hybrids between V. rotundifolia and Euvitis are sterile to a marked degree, fruit on hybrid vines being the exception.

After a complete survey of the characters that are found in Munson's vines and a very careful comparison of these with similar characters found associated with our hybrid vines, we come to the conclusion that Munson's vines are neither "false hybrids," nor true hybrids; that they are nothing but pure Muscadine vines.

The Viticultural Division of the U. S. Department of Agriculture in 1917 reports the production of a "supposed hybrid" vine in 1912 which vine undoubtedly should be classed with this group of so-called "false hybrids." This conclusion has been reached after a careful study of the description of this vine as given by Dearing (3) and a comparison of this with the hybrid vines that were produced at this station. The description reads as follows: "The next year (1912) one supposed hybrid was secured, a cross of Eden (female) and Flame Tokay (male) which has now fruited two years. While it is distinct from other Muscadine seedlings of Eden parentage, it is predominantly Muscadine in character and shows no resemblance to Flame Tokay except in the leaves."

We note from this description that this "supposed hybrid" differs from true hybrids in three prominent points, namely: (1) It shows no resemblance to the Vinifera parent except in the leaf, and just what this resemblance might be is not made clear. (2) The vine is predominantly Muscadine in character. (3) The vine has fruited for two years and no difference in its fruiting habit has been noticed from that of true Muscadine vines; in other words, this vine is normally fruitful and not practically sterile as are true hybrids.

Hybridization of Vitis rotundifolia with species of Euvitis produces true hybrids with varying intermediate and dominant characters, and not as has generally been supposed, "false hybrids" whose characters are chiefly those of Vitis rotundifolia.

SUMMARY

1. Hybrid vines, derived from Vitis rotudifolia and species of Euvitis, generally are more or less intermediate in character. This conclusion is based on a careful study of the characters of hybrid vines which have been derived from Vitis rotundifolia and the following Euvitis

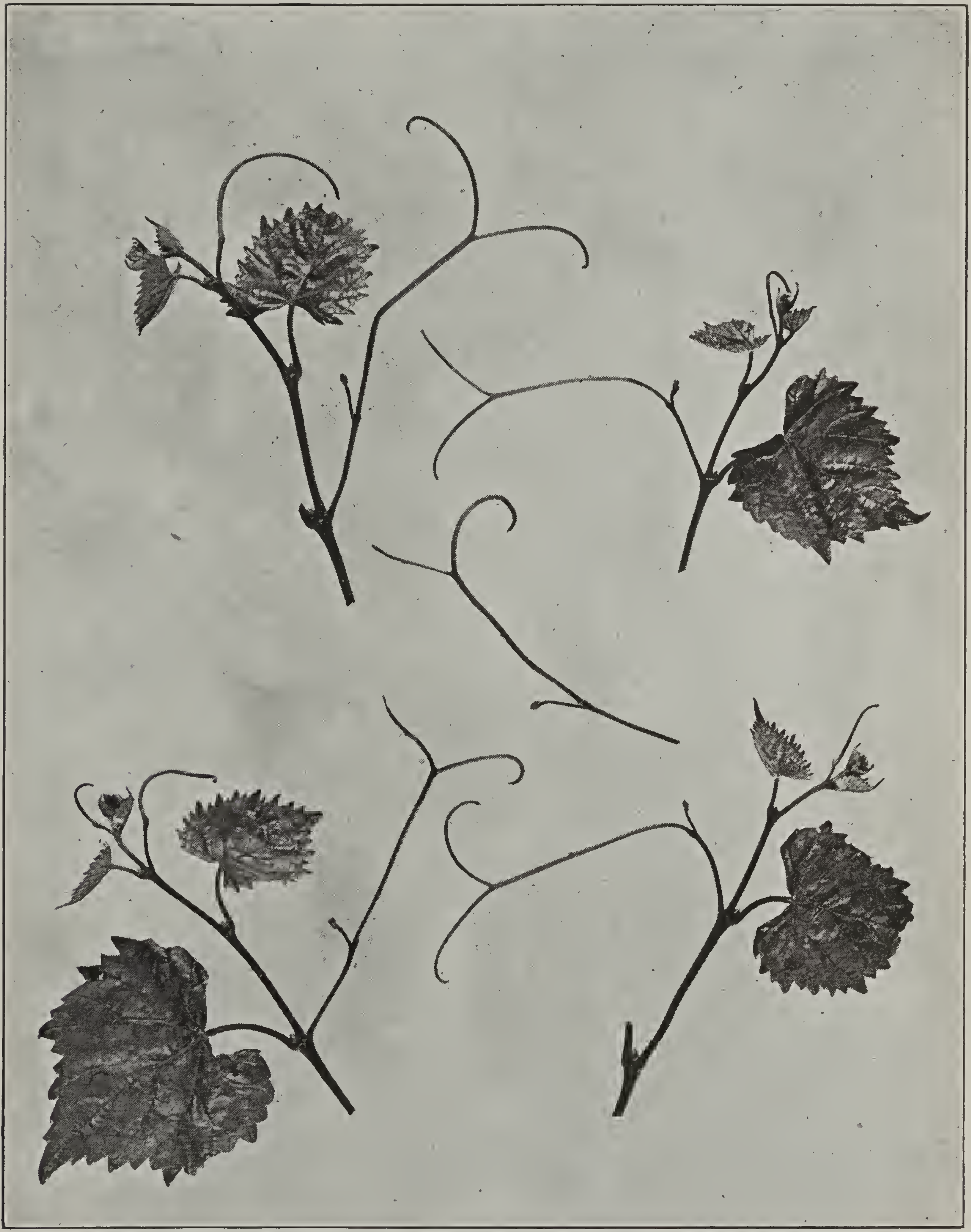

Fig. 31. Selected shoots of a Rotundifolia vine showing that even trifid tendrils may be found within this species. This trifid tendril was first found on a wild (Oberlin) vine and later has been transmitted to a number of its progeny. Reduced.

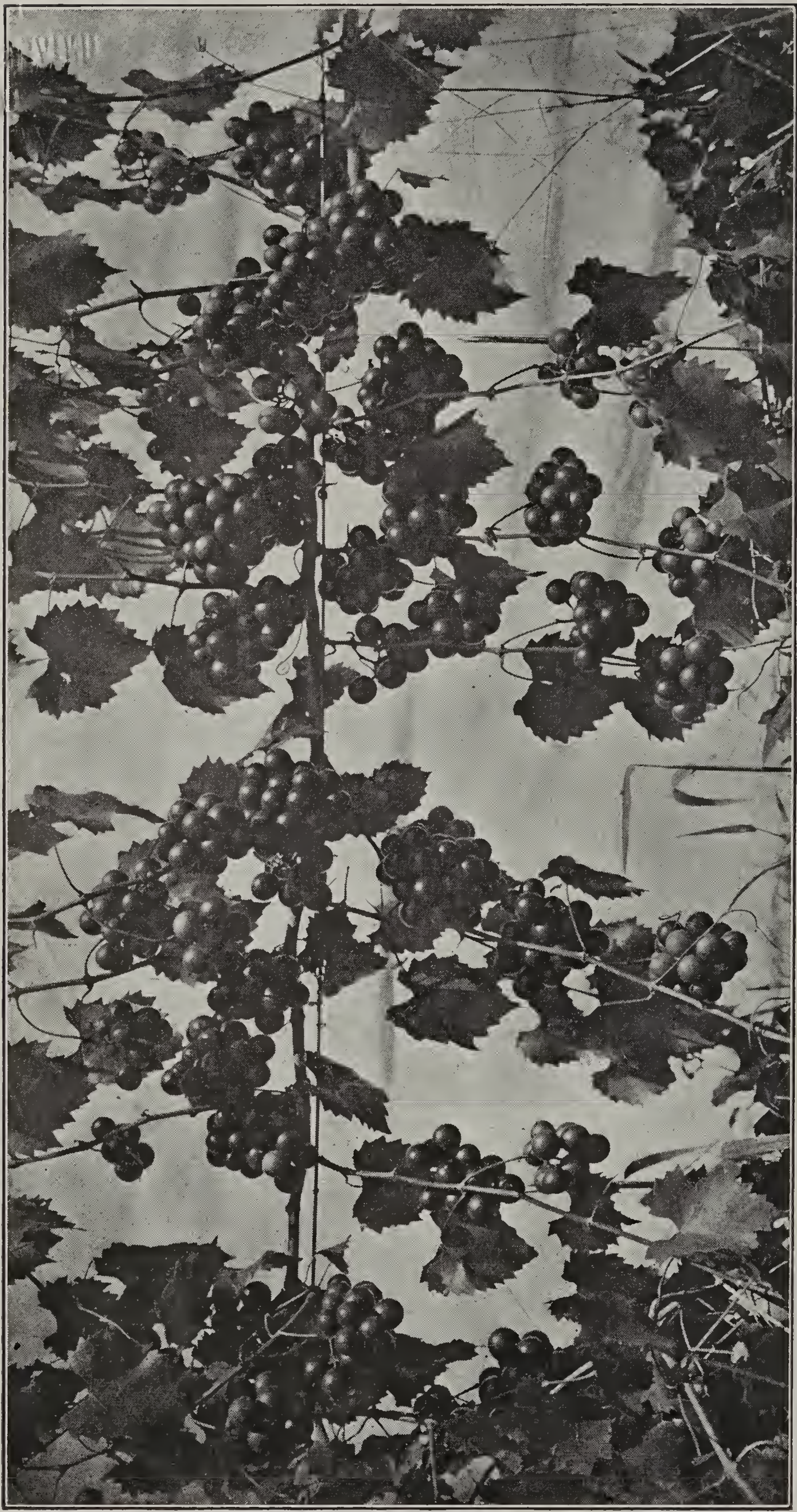

Fig. 32. A photograph of a branch of a Luola vine (V. rotundifolia), showing that fairly large clusters of fruit may be produced on Rotundifolia vines. Reduced.

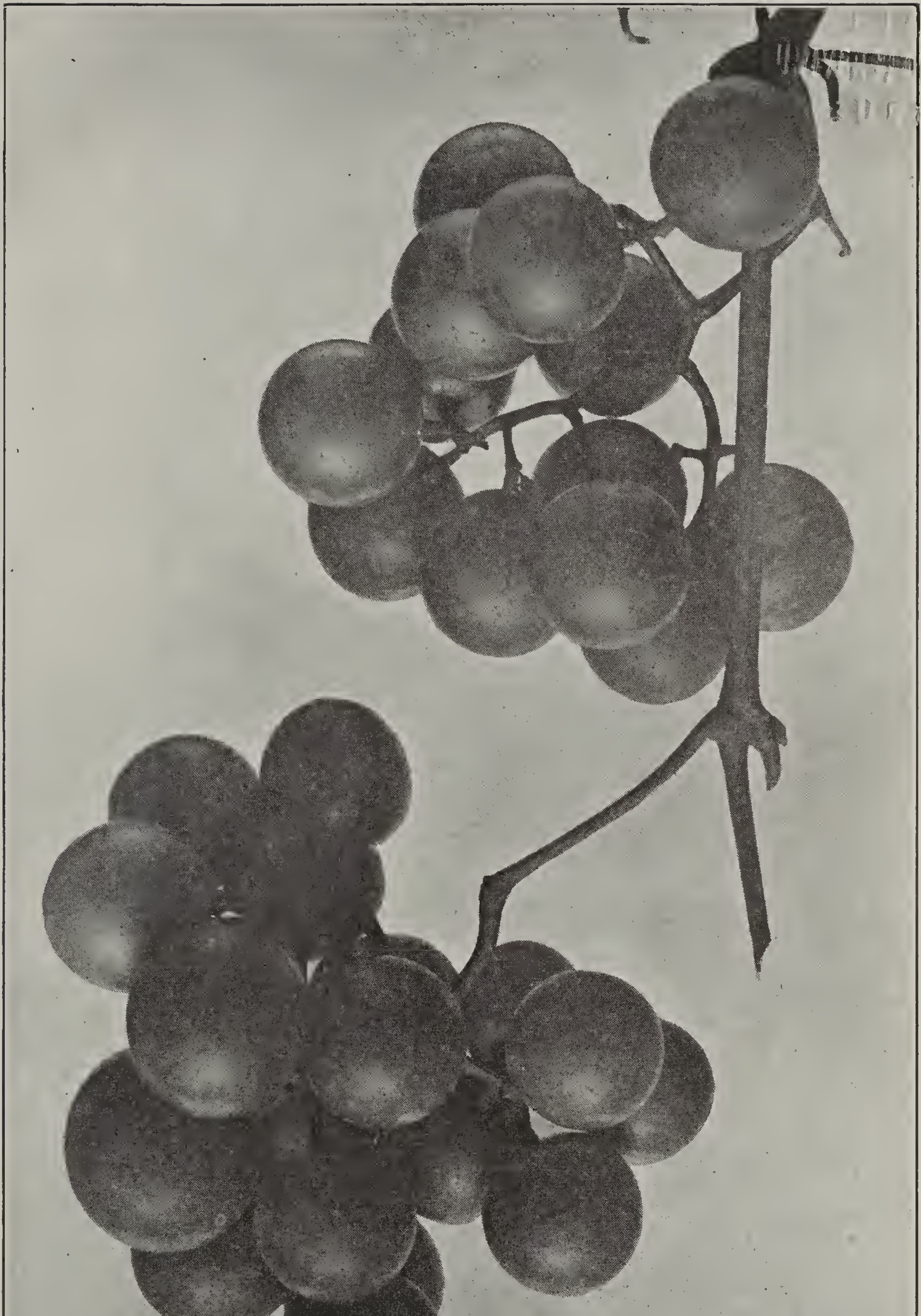

Fig. 33. Two clusters of fruit from a Rotundifolia (Oberlin) vine which shows that all Rotundifolia fruit-clusters are not small. Natural size.

species: V. vinifera, V. labrusca, V. bourquiniana, V. cordifolia and the hybrid variety Winchell.

2. The Muscadine group when hybridized with species of Euvitis is not as prepotent in regard to its external or visible characteristics as has generally been supposed.

3. The hybrids which are derived from Vitis rotundifolia and species of Euvitis are almost sterile and this sterility is due mainly to hybridization. The perfect hermaphroditic forms are sterile from this cause alone, while the imperfect hermaphroditic and the staminate vines are sterile because of the double phenomenon of sex or intersexualism and hybridization.

4. The so-called "false hybrids" which have been reported at various times in horticultural literature and which are said to be hybrids between Muscadine vines and species of Euvitis are not hybrids but straight seedlings of the supposedly dominant parent species, Vitis rotundifolia and Vitis munsoniana.

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